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#846

February 19, 2019

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5001 Campus Drive
College Park, MD 20740-3835

Attention: Dr. Paulette Gaynor
Re: GRAS Notification – Rebaudioside M

Dear Dr. Gaynor:

GRAS Associates, LLC, acting as the Agent for GLG Life Tech Corporation (Canada), is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notification for *Rebaudioside M*. Along with GLG's determination of safety, an Expert Panel of qualified persons was assembled to assess the composite safety information of the subject substance with the intended use as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into food in general, other than infant formulas and meat and poultry products. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

William J. Rowe
President
Agent for Blue California
GRAS Associates, LLC
27499 Riverview Center Blvd., Suite 212
Bonita Springs, FL 34134
wrowe@nutrasource.ca

Enclosure: GRAS Notification for GLG Life Tech Corporation –*Rebaudioside M*

GRAS Notification

of

Rebaudioside M
(≥95%)

Food Usage Conditions for General Recognition of Safety

on behalf of

GLG Life Tech Corporation

Suite 100-10271 Shellbridge Way
Richmond, B.C. V6X 2W8
Canada

2/19/19

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FOREWORD

GLG Life Tech Corporation ("GLG") based our Generally Recognized as Safe (GRAS) assessment on rebaudioside M primarily on the composite safety information, i.e., scientific procedures with corroboration from history of use. The safety/toxicity of steviol glycosides, history of use of steviol glycosides, and compositional details, specifications, and method of preparation of the subject ingredient were reviewed. In addition, a search of the scientific and regulatory literature was conducted through December 11, 2018, with particular attention paid to adverse reports, as well as those that supported conclusions of safety. Those references that were deemed pertinent to this review are listed in Part 7. The composite safety/toxicity studies, in concert with dietary exposure information, ultimately provide the specific scientific foundation for the GRAS conclusion.

At GLG's request, GRAS Associates, LLC ("GA") convened an Expert Panel to complete an independent safety evaluation of GLG's Festeviol™ Reb M 95 product. GLG's high purity Festeviol™ Reb M 95 preparation is produced through bioconversion of high purity rebaudioside A by genetically-modified *E. coli* or *Bacillus* expression systems, which is purified to yield a ≥95% rebaudioside M product. The purpose of the evaluation is to ascertain whether GLG's conclusion that the intended food uses of Festeviol™ Reb M 95 as described in Part 3 are generally recognized as safe, i.e., GRAS, under the intended conditions of use. In addition, GLG has asked GA to act as Agent for the submission of this GRAS notification.

PART 1. SIGNED STATEMENTS AND CERTIFICATION

A. Basis of Exclusion from the Requirement for Premarket Approval Pursuant to Subpart E of 170[1]

GLG has concluded that our high purity rebaudioside M product, referred to as "Festeviol™ Reb M 95" and "Festeviol™ RM 95," and which meets the specifications described below, is GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic (FD&C) Act. This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the intended conditions of food use for the designated high purity rebaudioside M preparation.

[1] See 81 FR 54960, 17 August 2016. Accessible at: https://www.gpo.gov/fdsys/pkg/FR-2016-08-17/pdf/2016-19164.pdf (Accessed 12/11/18).

Signed:

Agent for GLG

William J. Rowe
President and CEO
GRAS Associates, LLC
27499 Riverview Center Blvd.
Suite 212
Bonita Springs, FL 34134

Date: 2/18/19

B. Name and Address of Responsible Parties

GLG Life Tech Corporation
10271 Shellbridge Way
Suite 100
Richmond, B.C. V6X 2W8
Canada

As the Responsible Party, GLG accepts responsibility for the GRAS conclusion that has been made for our high purity rebaudioside M (≥ 95%) preparation, Festeviol™ RM 95, as described in the subject safety evaluation; consequently, the purified steviol glycosides preparations having acceptable steviol glycosides compositions which meet the conditions described herein, are not subject to premarket approval requirements for food ingredients.

C. Common Name and Identity of Notified Substance

The common name of the ingredient to be used on food labels is “high purity rebaudioside M.” GLG also plans to market our high purity rebaudioside M preparations under the trade names “Festeviol™ RM 95” and “Festeviol™ Reb M 95.”

D. Conditions of Intended Use in Food

GLG’s Festeviol™ high purity rebaudioside M (≥ 95%) preparation is intended for use as a general-purpose sweetener in foods, excluding meat and poultry products and infant formulas, at levels determined by Current Good Manufacturing Practices (CGMP).

E. Basis for GRAS Conclusion

Pursuant to 21 CFR 170.30(a) and (b), GLG's Festeviol™ high purity rebaudioside M (≥ 95%) preparation has been concluded to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

High purity rebaudioside M is not subject to premarket approval requirements of the FD&C Act based on GLG's conclusion that the substance is GRAS under the conditions of its intended food uses.

GLG certifies, to the best of our knowledge, that this GRAS notice is a complete, representative, and balanced assessment that includes all relevant information, both favorable and unfavorable, available and pertinent to the evaluation of safety and GRAS status of high purity rebaudioside M.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notice will be maintained at the offices of GLG Life Tech Corporation, 10271 Shellbridge Way, Suite 100, Richmond, BC V6X 2W8 Canada, and will be made available during customary business hours.

GLG certifies that no data or information contained herein are exempt from disclosure under the Freedom of Information Act (FOIA). No non-public, safety-related data were used by the Expert Panel to reach a GRAS conclusion.

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Chemical Identity of Ingredient

"Rebaudioside M" is the common or usual name of the non-nutritive sweetener derived from high purity rebaudioside A by genetically-modified *E. coli* or *Bacillus*. The compositional features of Festeviol™ RM 95 are described in more detail in this section. "Rebaudioside M" and "Reb M" are the terms used by GLG in referring to the notified substance. The preparation is also marketed as "Festeviol™ Reb M 95" and "Festeviol™ RM 95."

The general chemistry of rebaudioside M has previously been reviewed in a number of GRAS Notifications (GRN), including GRN 473[2] (PureCircle, 2013b), GRN 512 (GLG, 2014), GRN 667 (Blue California, 2016), GRN 744 (PureCircle, 2018a), and GRN 745 (PureCircle, 2018b).

No known toxins have been identified in stevia or stevia-derived products.

[2] GRN 473 was originally filed as Rebaudioside X. The FDA "no questions" letter clarified the nomenclature of the subject ingredient as Rebaudioside M.

1. Chemistry of Rebaudioside M

Rebaudioside M is a minor, naturally occurring steviol glycoside obtained from the leaves of *Stevia rebaudiana* Bertoni. It is reported to be 160-500 times sweeter than sucrose. Similar to the other steviol glycosides, Reb M is an *ent*-kaurane diterpene glycoside with a steviol backbone. Unlike the other steviol glycosides, Reb M has two 2-O-ß-D-glucopyranosyl-3-O-ß-D-glucopyranosyl-ß-D-glucopyranosyl units, an ether at position C-13 and an ester at position C-19 (Chaturvedula et al., 2013; Prakash et al., 2014).

Chemical name: 13-[(O-ß-D-Glucopyranosyl-(1-2)-O-[ß-D-glucosylpyranosyl-(1-3)]-ß-D-glucosylpyranosyl)oxy]-kaur-16-en-18-oic acid (4-)-O-ß-D-glucosylpyranosyl-(1-2)-O-[ß-D-glucosylpyranosyl-(1-3)]-ß-D-glycosylpyranosyl ester

Synonyms: Rebaudioside M, Reb M, Rebaudioside X, Reb X

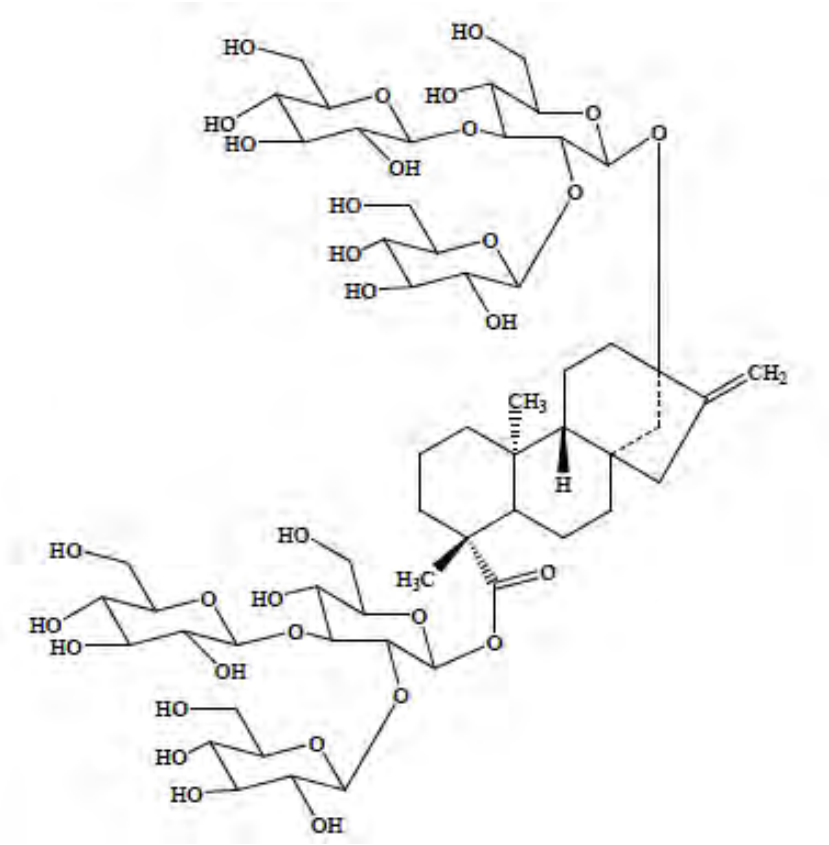
Chemical formula: $C_{56}H_{90}O_{33}$

Molecular weight: 1291.29 daltons

CAS Number: 1220616-44-3

The chemical structure of rebaudioside M is presented in Figure 1.

Figure 1. Chemical Structure of Rebaudioside M[a]



[a] From Chaturvedula et al. (2013)

2. Chemistry of the Bacterial Vectors

GLG's manufacturing process for its high purity rebaudioside M Festeviol™ RM 95 preparation uses glucosyltransferase and sucrose synthase enzymes produced by either an *E. coli* or *Bacillus* (consisting of *Bacillis brevis* and *Bacillis licheniformis*) expression system to carry out the biotransformation process.

a. *E. coli* Expression System

E. coli is a Gram-negative straight rod bacteria. Non-pathogenic strains serve an important role in suppressing the growth of harmful bacteria in the human body as well as synthesizing vitamins, whereas pathogenic strains can cause infection in the enteric, urinary, pulmonary, and nervous systems (UniProt, 2018c).

E. coli strains K-12 and B (also referred to as BL21) are considered "workhorse" organisms, and are used in many research laboratories worldwide as well as for industrial scale expression of recombinant proteins (UniProt, 2018b; Marisch et al., 2013).

Previous GRNs have been reviewed by FDA for which *E. coli* is the host microorganism. Two of these notifications detailed the production of rebaudioside M using genetically-modified strains of *E. coli* LE1B09 and *E. coli* K-12 in (PureCircle, 2018b) and GRN 780 (Tate and Lyle, 2018), respectively.

GLG uses non-pathogenic and non-toxigenic strains of *E. coli* K-12 and *E. coli* B to express the two uridine diphosphate (UDP)-glucosyltransferase enzymes and sucrose synthase enzyme that are used to produce rebaudioside M. Furthermore, GLG's *E. coli* production strains meet the criteria for Biosafety Level 1 organisms, as outlined by the National Institutes of Health (NIH, 2016).

The major genetic modification of these strains is the directional deletion of certain genetic materials in order to accommodate the introduction of exogenous deoxyribonucleic acid (DNA) materials for the production of the targeted enzymes, thereby making the resulting transformants more stable in achieving high density fermentation with optimum growth and protein expression profile. The systems GLG has developed demonstrate stable genetic transformation and can express the targeted proteins efficiently. The plasmids used in this particular project are pNYK and pNYY.

UDP-glucosyltransferase 1, UDP-glucosyltransferase 2, and sucrose synthase are produced by *E. coli* K-12 and B strains by fermentation of the genetically-engineered *E. coli* under standard culture conditions. After fermentation, the reaction solution is heat-treated to disrupt the reaction. The enzymes are then obtained through modular membrane filtration of the fermentation broths to remove impurities.

b. *Bacilli* Expression System

Bacilli are Gram-positive, rod-shaped, spore-forming bacteria. While certain species are pathogens, the majority of *Bacillus* species are "harmless saprophytes." *Bacilli* are used in a number of medical, pharmaceutical, agricultural, and industrial applications, where they can be used to produce enzymes, antibiotics, and metabolites (UniProt, 2018a; Turnbull, 1996).

Bacillus brevis and *Bacillus licheniformis* have been widely used in the production of food and pharmaceutical raw materials, and have been granted GRAS status by FDA. No harmful substances coming from these strains have been reported.

Previous GRNs have been reviewed by FDA for which *Bacilli* are the host microorganisms. In addition, 4 GRNs were submitted regarding steviol glycosides preparations manufactured using genetically-modified *Bacilli*: GRN 375 (Toyo Sugar Refining Co., 2011); GRN 448 (Daepyung, 2012); GRN 607 (PureCircle, 2015); and GRN 662 (PureCircle, 2016).

GLG uses non-pathogenic and non-toxigenic strains of *Bacillus brevis* and *Bacillus licheniformis* to express the two UDP glucosyltransferase enzymes and sucrose synthase enzyme that are used to produce rebaudioside M. Furthermore, GLG's *Bacillus brevis* and *Bacillus licheniformis* production

strains meet the criteria for Biosafety Level 1 organisms, as outlined by the National Institutes of Health (NIH, 2016).

The major genetic modification of these strains is the directional deletion of certain genetic materials in order to accommodate the introduction of exogenous DNA materials for the production of the targeted enzymes, thereby making the resulting transformants more stable in achieving high density fermentation with optimum growth and protein expression profile. The systems GLG has developed demonstrate stable genetic transformation and can express the targeted proteins efficiently. The plasmids used in this particular project are pNYK and pNYY.

UDP-glucosyltransferase 1, UDP-glucosyltransferase 2, and sucrose synthase are produced by *Bacillus brevis* and *Bacillus licheniformis* strains by fermentation of the genetically-engineered *Bacillus* under standard culture conditions. After fermentation, the reaction solution is heat-treated to disrupt the reaction. The enzymes are then obtained through modular membrane filtration of the fermentation broths to remove impurities.

B. Manufacturing Processes

GLG manufactures Festeviol™ RM95 in a process that uses genetically-modified bacterial strains that produce glucosyltransferase and sucrose synthase enzymes that facilitate the transfer of glucose to small molecules *via* glycosidic bonds.

1. Bioconversion Process

The recombinant *E. coli* or *Bacillus* strains containing the target enzymes are precultured as a seed cultivation and then transferred to a large fermenter for scale-up. The glucosyltransferase and sucrose synthase enzymes produced in the host bacteria cells are secreted into the culture supernatant. After fermentation, the reaction solution is heat-treated to disrupt the reaction. The enzymes are then obtained through modular membrane filtration of the fermentation broth to remove impurities.

GLG uses a purified steviol glycosides extract (> 95% total steviol glycosides) starting material, which is derived from *Stevia rebaudiana* leaves. Product specifications for the > 95% steviol glycosides starting material are provided in Appendix 1.

The stevia extract, sucrose, and prepared enzymes are mixed in the fermentation tank and bioconversion is allowed to proceed. After the bioconversion is completed, the reaction solution is centrifuged, and the supernatant is collected for downstream purification processes.

2. Extraction & Purification

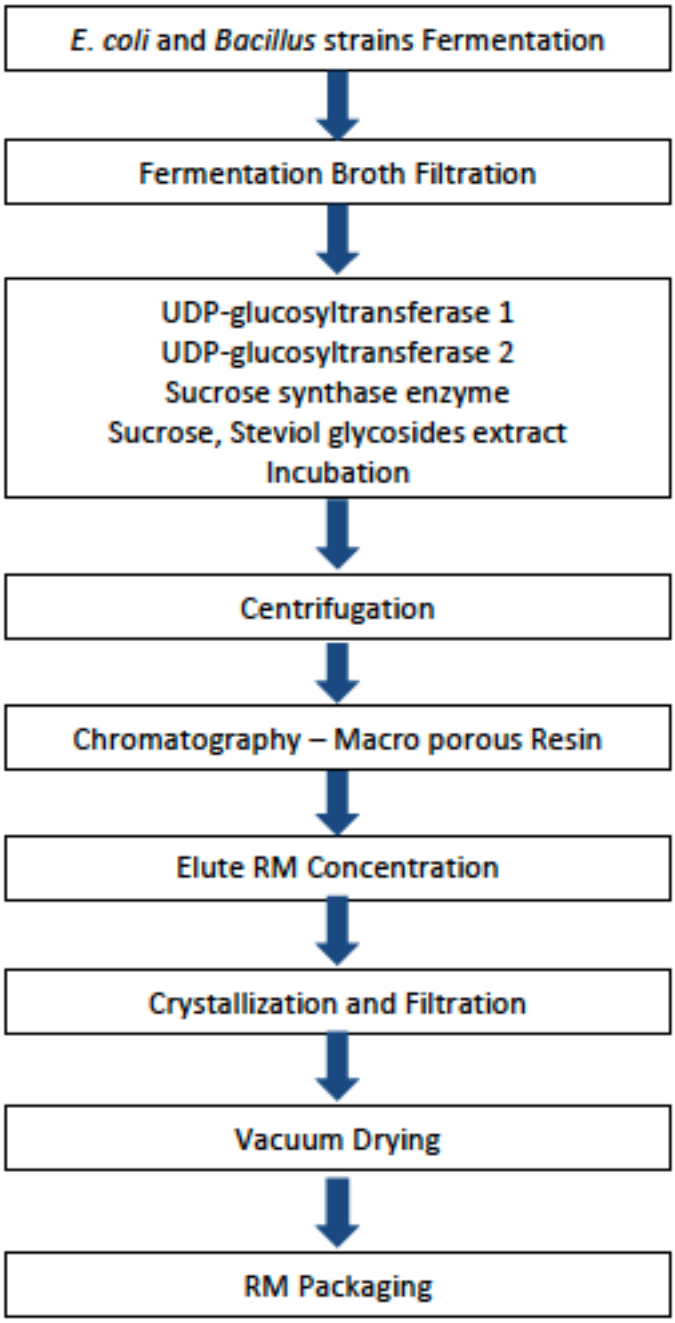
The supernatant from the bioconversion process, described above, is filtered through columns packed with macroporous adsorption resin. The ion exchange resin concentrates the rebaudioside M, which is eluted with food grade ethanol and spray dried. The spray dried rebaudioside M is

dissolved in ethanol, crystallized, and filtered. The crystallization and drying processes are repeated one or several more times using ethanol to obtain high purity rebaudioside M. The rebaudioside M crystals are finally separated by plate filtration and vacuum dried to obtain the finished dry powder product, Festeviol™ RM 95.

The manufacturing process is summarized in a flow chart provided in Figure 2.

The food grade ethanol used in the macro porous resin chromatography and crystallization processes complies with Food Chemicals Codex (FCC) 8th Edition specifications. The ion exchange resin used in the manufacturing process complies with 21 CFR 173.65. GLG's Festeviol™ RM 95 is prepared in accordance with CGMP. Certificates of analyses and/or specifications for these materials are provided in Appendix 1.

Figure 2. Flow Chart of Manufacturing Process for GLG's Festeviol™ RM 95



C. Product Specifications

1. JECFA Specifications for Steviol Glycosides

The compositions of extracts of *Stevia rebaudiana* Bertoni depend upon the compositions of the harvested leaves, which are, in turn, influenced by soil, climate, and the manufacturing process itself (FAO, 2007).

In the most recent Joint FAO/WHO Expert Committee on Food Additives (JECFA) monograph, published in 2017 (FAO, 2017), steviol glycosides specifications were modified to include a minimum requirement of not less than 95% total steviol glycosides, on a dry basis, "determined as the sum of all compounds containing a steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni."

JECFA's 2017 monograph describes steviol glycosides as white-to-yellow powders that are odorless or have a slight characteristic odor and exhibit a sweetness that is 200-300 times greater than that of sucrose. The ingredient must consist of a minimum of 95% total steviol glycosides, as defined above. The steviol glycosides are freely soluble in a 50:50 mixture of ethanol and water, and the 1 in 100 solutions exhibit pH values between 4.5 and 7.0. The product should not have more than 1% ash, with no more than a 6% loss on drying at 105°C after 2 hours. Any residual methanol levels should not exceed 200 mg per kg, and ethanol residues should not exceed 5,000 mg per kg. Arsenic and lead levels should not exceed 1 mg per kg. Microbiological criteria have also been established, with specifications of no more than 1,000 colony forming units (CFU) per Todorov et al. (2011)total plate count, not more than 200 CFU per g yeasts and molds, and *E. coli* and *Salmonella* negative in 1 g and 25 g, respectively.

2. Specifications for GLG's Festeviol™ High Purity Rebaudioside M Preparation and Supporting Methods

GLG's Festeviol™ RM 95 preparation meets or exceeds JECFA recommendations, while also complying with Food Chemicals Codex (FCC, 2010) specifications for rebaudioside A as a consumable human food substance. The compositions of five non-consecutive batches of GLG's Festeviol™ RM 95 are compared with the JECFA and FCC specifications in Table 1.

Table 1. Specifications for GLG's Festeviol™ RM 95

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Rebaudioside A	**GLG's Specifications** for Festeviol™ RM 95	Festeviol™ RM 95 Representative Batches				
				Batch Number	Batch Number	Batch Number	Batch Number	Batch Number
Appearance Form	Powder	Crystal, granule or powder	Powder	Powder	Powder	Powder	Powder	Powder
Appearance Color	White to light Yellow	White to off-white	White	White	White	White	White	White
Solubility	Freely soluble in water: ethanol (50:50)	Freely soluble in water:ethanol (50:50)	NS[c]	NS	NS	NS	NS	NS
Purity (HPLC Area)	≥95% Steviol Glycosides	≥ 95% Reb A	≥ 95.0% Reb M ≥95% Total Steviol Glycosides	96.29% 98.69%	96.12% 98.65%	96.62% 98.43%	96.70% 98.90%	96.32% 98.78%
Residual Ethanol	NMT 5,000 mg/kg	NMT 0.5%	≤ 5,000 ppm	2,321.8 ppm	1,265.4 ppm	1,873.8 ppm	1,678.3 ppm	1,587.3 ppm
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	≤ 200 ppm	38.6 ppm	78.9 ppm	91.4 ppm	58.9 ppm	83.5 ppm
Loss on Drying	NMT 6.0%	NMT 6.0%	≤ 6.0%	2.68%	2.32%	2.92%	2.28%	2.53%
pH, 1% Solution	4.5-7.0	4.5-7.0	4.5-7.0	5.2	5.6	5.1	5.7	5.5
Total Ash	NMT 1%	NMT 1%	≤ 1.0%	0.05%	0.07%	0.08%	0.07%	0.09%
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	≤ 1.0 ppm	0.06 ppm	0.02 ppm	0.03 ppm	0.05 ppm	0.04 ppm
Lead	NMT 1 mg/kg	NMT 1 mg/kg	≤ 1.0 ppm	0.07 ppm	0.03 ppm	0.05 ppm	0.04 ppm	0.02 ppm
Cadmium	NS	NS	≤ 1.0 ppm	0.01 ppm	0.03 ppm	0.01 ppm	0.02 ppm	0.01 ppm
Mercury	NS	NS	≤ 1.0 ppm	0.02 ppm	0.01 ppm	0.01 ppm	0.01 ppm	0.03 ppm
Total Plate Count (cfu/g, max)	NMT 1,000	NS	< 1,000	< 10	< 10	< 10	< 10	< 10
Yeast & Mold (cfu/g, max)	NMT 200	NS	< 100	< 10	< 10	< 10	< 10	< 10
E. coli (mpn/g)	Negative in 1 g	NS	<3	< 3	< 3	< 3	< 3	< 3
Salmonella spp.	Negative in 25 g	NS	Negative in 25 g	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	NS	NS	<10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g

[a] Prepared at 84th JECFA (2017)
[b] Rebaudioside A Monograph. Food Chemicals Codex (7th Ed.). (FCC, 2010)
[c] GLG has not set solubility specifications for Festeviol™ RM 95; however, solubility data presented in Appendix 4 demonstrate that the preparation meets JECFA recommendations for steviol glycosides.
Andersen et al. (2013)= not specified; NMT = not more than

In addition to the presentation of key specifications found in Table 1 for comparison with generally accepted purity standards, certificates of analysis for five representative lots of Festeviol™ RM 95 are provided in Appendix 2. The chromatograms for representative lots of Festeviol™ RM 95 are provided in Appendix 3.

GLG has also tested the solubility of five representative lots of material in water:ethanol (50:50). All five lots were found to be freely soluble, demonstrating that GLG's Festeviol™ RM 95 preparation meets JECFA recommendations for steviol glycosides. A solubility study report is provided in Appendix 4.

GLG analyzes its Festeviol™ RM 95 preparation by high performance liquid chromatography (HPLC), following the JECFA's 'Steviol Glycosides from *Stevia rebaudiana* Bertoni' monograph (FAO, 2017). Test reports for analysis of pesticide residues and residual protein in a representative lot of Festeviol™ RM 95 are located in Appendix 5 and Appendix 6, respectively. The collection of these reports demonstrates that the substance is well characterized and meets the established purity criteria.

D. Physical or Technical Effect

GLG determined the relative sweetness of Festeviol™ RM 95 to be 200 X sweeter than sucrose by organoleptic comparison following the method outlined in Appendix 7.

E. Stability

1. Stability Data on Steviol Glycosides and Rebaudioside M

Steviol glycosides have been reported to be stable over the pH range 3-9 and can be heated at 100°C for 1 hour, but, at pH levels greater than 9, they rapidly decompose (Kinghorn, 2002). In previously submitted GRAS Notifications, GRN 252 (Merisant, 2008), GRN 253 (Cargill, 2008), and GRN 304 (Sunwin/WILD, 2010) reported stability data indicating that rebaudioside A is stable under the intended conditions of use.

The stability of purified rebaudioside M has previously been reviewed in detail in a number of GRNs, including GRN 473[3] (PureCircle, 2013b), GRN 512 (GLG, 2014), GRN 667 (Blue California, 2016), GRN 744 (PureCircle, 2018a), GRN 745 (PureCircle, 2018b), and GRN 780 (Tate and Lyle, 2018).

Furthermore, in the over 50 GRAS Notifications that have been submitted to FDA to date for steviol glycosides, the presented stability data have supported the position that steviol glycosides are stable and well-suited for the intended uses in foods.

[3] GRN 473 was originally filed as Rebaudioside X. The FDA "no questions" letter clarified the nomenclature of the subject ingredient as Rebaudioside M.

2. Stability Data for GLG's Festeviol™ RM 95

GLG conducted a 6-month stability study of five lots of Festeviol™ RM 95. The samples were stored at 40°C ± 2°C at a relative humidity of 75% ± 5%. Festeviol™ RM 95 was observed to be stable over the course of the accelerated stability study, as demonstrated in Table 2.

Table 2. Festeviol™ RM 95 Storage Stability Data

Festeviol™ RM 95 Lot#						
Duration	Reb M (%)	Total Steviol Glycosides (%)	Total Plate Count (cfu/g)	*Salmonella*	*E. coli*	*Staphylococcus*
t=0	96.70	98.90	< 10	Negative	Negative	Negative
1 month	96.60	98.82	< 10	Negative	Negative	Negative
2 months	96.32	98.37	< 10	Negative	Negative	Negative
3 months	96.12	98.81	< 10	Negative	Negative	Negative
4 months	95.92	98.62	< 10	Negative	Negative	Negative
5 months	96.20	98.52	< 10	Negative	Negative	Negative
6 months	95.71	98.43	< 10	Negative	Negative	Negative
Festeviol™ RM 95 Lot#						
Duration	Reb M (%)	Total Steviol Glycosides (%)	Total Plate Count (cfu/g)	*Salmonella*	*E. coli*	*Staphylococcus*
t=0	96.32	98.78	< 10	Negative	Negative	Negative
1 month	96.53	98.89	< 10	Negative	Negative	Negative
2 months	96.48	98.82	< 10	Negative	Negative	Negative
3 months	96.01	98.78	< 10	Negative	Negative	Negative
4 months	95.78	98.65	< 10	Negative	Negative	Negative
5 months	96.10	98.43	< 10	Negative	Negative	Negative
6 months	95.58	98.52	< 10	Negative	Negative	Negative
Festeviol™ RM 95 Lot#						
Duration	Reb M (%)	Total Steviol Glycosides (%)	Total Plate Count (cfu/g)	*Salmonella*	*E. coli*	*Staphylococcus*
t=0	96.62	98.43	< 10	Negative	Negative	Negative
1 month	96.73	98.98	< 10	Negative	Negative	Negative
2 months	96.58	98.59	< 10	Negative	Negative	Negative
3 months	96.32	98.76	< 10	Negative	Negative	Negative
4 months	95.89	98.82	< 10	Negative	Negative	Negative
5 months	95.67	98.47	< 10	Negative	Negative	Negative
6 months	95.45	98.59	< 10	Negative	Negative	Negative

Festeviol™ RM 95 Lot#						
Duration	Reb M (%)	Total Steviol Glycosides (%)	Total Plate Count (cfu/g)	*Salmonella*	*E. coli*	*Staphylococcus*
t=0	96.12	98.65	< 10	Negative	Negative	Negative
1 month	96.35	98.76	< 10	Negative	Negative	Negative
2 months	96.48	98.58	< 10	Negative	Negative	Negative
3 months	96.07	98.81	< 10	Negative	Negative	Negative
4 months	95.82	98.78	< 10	Negative	Negative	Negative
5 months	96.01	98.78	< 10	Negative	Negative	Negative
6 months	95.92	98.73	< 10	Negative	Negative	Negative
Festeviol™ RM 95 Lot#						
Duration	Reb M (%)	Total Steviol Glycosides (%)	Total Plate Count (cfu/g)	*Salmonella*	*E. coli*	*Staphylococcus*
t=0	96.29	98.69	< 10	Negative	Negative	Negative
1 month	96.32	98.76	< 10	Negative	Negative	Negative
2 months	96.10	98.87	< 10	Negative	Negative	Negative
3 months	96.28	98.59	< 10	Negative	Negative	Negative
4 months	95.98	98.63	< 10	Negative	Negative	Negative
5 months	95.86	98.51	< 10	Negative	Negative	Negative
6 months	95.62	98.46	< 10	Negative	Negative	Negative

The stability data in the scientific literature for stevioside, the JECFA report, and the extensive stability testing for the structurally similar rebaudioside A as presented by Merisant, Cargill, and Sunwin & WILD Flavors, along with stability data for purified rebaudioside M presented in 6 previous GRNs in concert with GLG's accelerated stability testing results, support the position that GLG's Festeviol™ RM 95 preparation is well-suited for the intended food uses.

In addition, GLG claims a 2-year shelf life for Festeviol™ RM 95.

PART 3. DIETARY EXPOSURE

The subject GLG Festeviol™ RM 95 preparation is intended to be used as a table top sweetener and general-purpose non-nutritive sweetener in various foods other than infant formulas and meat and poultry products. The intended use will be as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19).[4] The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts

[4] Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.

of GLG's Festeviol™ RM 95 preparation to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.[5]

A. Estimate of Dietary Exposure to the Substance

The potential dietary intake of sugar-replacement sweeteners, including steviol glycosides, has been evaluated in a number of published studies (FSANZ, 2008; WHO, 2003; Renwick, 2008) or GRAS Notifications (Merisant, 2008; BioVittoria, 2009). These are summarized in Appendix 8. In GRAS notification 301, a simplified estimate was proposed to, and accepted by, FDA based on the estimates of exposure in "sucrose equivalents" (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would consume a maximum of 9.9 mg per kg body weight (bw) per day for any population subgroup.

The estimated sweetness intensity for GLG's Festeviol™ RM 95 is approximately 200-fold that of sucrose (Part 2.D). Therefore, the highest 90th percentile consumption by any population subgroup of GLG's Festeviol™ RM 95 preparation would be approximately 4.95 mg per kg bw rebaudioside M per day. Based on an estimate that rebaudioside M consists of approximately 25% steviol equivalents,[6] the consumption would be less than 1.22 mg per kg bw per day on a steviol equivalents basis for any population group. These calculations are summarized in Table 3.

Table 3. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of Rebaudioside M

Population Group	Intakes of Sweeteners (mg sucrose/kg bw/day)[a]		Calculated Intake of Reb M (mg/kg bw/day)[b]		Calculated Intake of Reb M as Steviol Equivalents (mg/kg bw/day) [c]	
	Low	High	Low	High	Low	High
Healthy Population	255	675	1.28	3.38	0.31	0.83
Diabetic Adults	280	897	1.40	4.49	0.34	1.10
Healthy Children	425	990	2.13	4.95	0.52	1.22
Diabetic Children	672	908	3.36	4.54	0.83	1.12

[a] From Renwick (2008)
[b] Calculated by dividing the sucrose intake by the minimum average relative sweetness value of 200 for Festeviol™ RM 95.
[c] Calculated based on the ratio of molecular weights of Reb M and steviol.

[5] See 21 CFR 182.1(b)(1).
[6] Calculated as percent of molecular weight of steviol to molecular weight of rebaudioside M.

The values in Table 3 assume GLG's Festeviol™ RM 95 preparation constitutes the entire sweetener market, which makes these estimates extremely conservative since the likelihood of that occurrence is minimal. For the general healthy adult population, the estimated maximum intake of rebaudioside M is 3.38 mg per kg bw per day, or 0.83 mg per kg steviol equivalents. For healthy children, the estimated maximal intake is 4.95 mg per kg bw per day, or 1.22 mg per kg as steviol equivalents. In all population groups, the estimated daily intake of Festeviol™ RM 95, expressed as steviol equivalents, is well below the JECFA-established acceptable daily intake (ADI) of 4.0 mg per kg bw per day steviol equivalents.

B. Estimated Dietary Exposure to Any Other Substance That is Expected to be Formed In or On Food

This section is not applicable to GLG's Festeviol™ RM 95 product, which would be chemically stable under conditions of use.

C. Dietary Exposure to Contaminants or Byproducts

While a recent publication by Kumari et al. (2016) investigated the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Antioxidant Capacity (TAC) in *S. rebaudiana* leaf --- and the observed activity has been attributed to naturally-occurring phytochemicals such as phenolics, flavonoids, and pigments in the plant --- the study has minimal relevance with regard to the safety considerations of highly purified stevia extract, of which ≥ 95% consists of the most familiar steviol glycosides and their glucosylated steviosides. These phytochemical contaminants, if present, are in low amounts, and were likely similarly present in purified test materials that were used in the toxicology studies summarized in Appendix 9.

Furthermore, no concerns regarding dietary exposure to contaminants or byproducts have been raised by expert regulatory bodies, including the World Health Organization/Joint FAO/WHO Expert Committee on Food Additives (WHO/JECFA), European Food Safety Authority (EFSA), Food Standards Australia New Zealand (FSANZ), and FDA, since JECFA's first steviol glycosides review was performed in 2000 (WHO, 2000).

PART 4. SELF-LIMITING LEVELS OF USE

It has been well-documented in the published literature that the use of steviol glycosides is self-limiting due to organoleptic factors and consumer taste considerations (Kochikyan et al., 2006; Carakostas et al., 2008; Brandle et al., 1998; Prakash et al., 2008; Gupta et al., 2016; Gerwig et al., 2016). These organoleptic factors include bitterness and astringency, as well as a lingering metallic aftertaste (Gerwig et al., 2016).

PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

A. Other Information on Dietary Exposure

1. History of Traditional Medicinal and Human Food Use

Stevia has been used as a traditional medicine and sweetener by native Guarani tribes for centuries (Esen, 2016; Gerwig et al., 2016; Brusick, 2008; Brandle et al., 1998). Hawke (2003) reported that stevia is commonly used as a treatment for type 2 diabetes in South America. However, for its therapeutic effects, elevated doses in the range of 1 gram per person per day or more were reported to be necessary (Gregersen et al., 2004).

For about 30 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners (Raintree, 2012). It was previously reported that 40% of the artificial sweetener market in Japan had been stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Use of steviol glycosides as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand, and as a natural health product in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated that sales of stevia in the US reached $45 million (Newsday, 2006).

NewHope360 reported that the global market for stevia in 2014 was $347 million, and that is expected to increase to $565.2 million by 2020. In addition, consumption is expected to increase from 2014 levels of 5,100.6 tons to 8,506.9 tons by 2020 (NewHope360, 2015).

Most recently, Nutritional Outlook reported that Mintel data indicated a 48% increase in stevia-containing products over the last five years (Decker and Prince, 2018).

B. Summary of Regulatory History of Steviol Glycosides

Stevia-derived sweeteners are permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, these sweeteners have received food usage approvals in Mexico, Australia, New Zealand, Switzerland, France, Peru, Uruguay, Colombia, Senegal, Russia, Malaysia, Turkey, Taiwan, Thailand, Israel, Canada, and Hong Kong (EFSA, 2010; Watson, 2010; Health Canada, 2012). In the United States, steviol glycosides have been used as a dietary supplement since 1995 (Geuns et al., 2003a).

A brief overview of the most recent regulatory activity regarding steviol glycosides is presented below in Part 5.B. Sections 1-5; a more detailed historical overview is provided in Appendix 10.

1. U.S. Regulatory History

Based on available information from FDA's GRAS Notice Inventory website (FDA, 2018) as of November 28, 2018, FDA has issued 56 "no questions" letters on GRAS notices on rebaudioside

A, rebaudioside D, rebaudioside M, or steviol glycosides, including those undergoing enzyme treatment. A comprehensive list is provided in Table 10-1 in Appendix 10.

In addition, the Flavor and Extract Manufacturers Association (FEMA) includes nine steviol glycosides preparations, one of which is for an enzymatically modified stevia extract, on their GRAS lists.

2. Canadian Regulatory History

On November 30, 2012, Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods (Health Canada, 2012). In March 2014, Health Canada updated the List of Permitted Sweeteners (Lists of Permitted Food Additives) to include steviol glycosides in applications as a table-top sweetener and as an ingredient in a variety of foods, beverages, baked goods, meal replacement bars, condiments, and confectionary and gums (Health Canada, 2014). On January 15, 2016, Health Canada approved the use of rebaudioside M as a high-intensity sweetener under the same conditions as the previously approved steviol glycosides (Health Canada, 2016).

Most recently, Health Canada's Food Directorate has updated its List of Permitted Sweeteners to allow for the use of steviol glycosides as a sweetener in 'unstandardized snack bars,' including granola bars, cereal bars, fiber bars, and protein isolate-based bars (Health Canada, 2017b). Health Canada (2017a) also modified the List of Permitted Sweeteners to include "all the steviol glycosides in the Stevia rebaudiana Bertoni plant (stevia plant)."

3. European Regulatory History

An amendment to the European Union (EU) food additives regulation 231/2012, which became active on November 3, 2016, removed the previous requirement for stevia blends to contain at least 75% Reb A or stevioside. In addition, the updated regulation ---(EU) 2016/1814---now permits the following steviol glycosides in stevia blends: stevioside, rebaudiosides A, B, C, D, E, F and M, steviolbioside, rubusoside, and dulcoside (Searby, 2016).

The EFSA Panel of Food Additives and Nutrient Sources reviewed an application for glucosylated steviol glycoside preparations for use as a new food additive. The Panel concluded that the data supplied by the applicant were "insufficient to assess the safety" of the glucosylated steviol glycosides preparation. It should be noted that no safety concerns were raised by the EFSA Panel, and that their decision was based on the "limited" data provided in the dossier submitted by the applicant (EFSA, 2018).

Recently, JECFA updated the steviol glycosides specifications to include a minimum requirement of not less than 95% total steviol glycosides, on a dry basis, "determined as the sum of all compounds containing a steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni." Microbiological criteria were also

established, with specifications of no more than 1,000 CFU per g total plate count, not more than 200 CFU per g yeasts and molds, and *E. coli* and *Salmonella* negative in 1 g and 25 g, respectively (FAO, 2017).

4. Asian Regulatory History

No regulatory updates have been identified in recent years. The Asian regulatory history for steviol glycosides through 2014 is presented in Appendix 10.

5. Other Regulatory History

FSANZ called for submissions on permitting all minor steviol glycosides extracted from stevia leaf to be included in the definition of steviol glycosides in the Food Standards Code, noting that "[no] evidence was found to suggest that the proposed changes pose any public health and safety concerns." The submission period ended on December 19, 2016 (FSANZ, 2016b). Subsequently, on February 8, 2017, FSANZ approved a draft variation of the definition of steviol glycosides to include all steviol glycosides present in the *Stevia rebaudiana* leaf (FSANZ, 2017).

Most recently, FSANZ called for comments on the production of Reb M using enzymes derived from genetically modified yeast (*Pichia pastoris*). The comment period closed on August 31, 2018 (FSANZ, 2018b). Subsequently, on October 31, 2018, FSANZ approved a draft variation to include a reference to the production method (FSANZ, 2018a). The final variation for Reb M was approved on January 10, 2019, and subsequently published in the Commonwealth of Australia Gazette No. FSC 124 on January 23, 2019 (FSANZ, 2019).

PART 6. NARRATIVE

The biological, toxicological, and clinical effects of stevia and steviol glycosides have been extensively reviewed (Carakostas et al., 2008; Geuns et al., 2003a; Huxtable, 2002). Additionally---and as noted earlier---the national and international regulatory agencies have thoroughly reviewed the safety of stevia and its glycosides. Most notably, over the years, JECFA has evaluated purified steviol glycosides multiple times (WHO, 2000; WHO, 2006; WHO, 2007; WHO, 2008), and their findings have been summarized in Part 5.B.3. FSANZ (2008) also evaluated steviol glycosides for use in food. The JECFA reviews, as well as the other reviews completed before 2008, primarily focused on mixtures of steviol glycosides. These studies are summarized in Appendix 11.

Since the JECFA evaluation (WHO, 2008), FDA has received and not objected to over fifty GRAS notifications for steviol glycosides or enzyme modified steviol glycosides that have been submitted to FDA, as detailed in Table 10-1 in Appendix 10 (Perrier et al., 2018). In each case, FDA has agreed with the conclusions that steviol glycosides are GRAS based largely on the 0-4 mg per kg bw per day ADI on a steviol equivalence basis that was established by JECFA. A recent publication by Roberts et al. (2016) indicates that the ADI could be higher, as discussed further in Appendix 8. Among the GRAS notifications submitted to FDA, several assessed purified

preparations of rebaudioside A, and they were supported by additional toxicology and clinical studies that are summarized in Appendix 12.

Because of their sweetness characteristics, steviol glycosides have viable uses as a non-nutritive sweetener in foods.[7] Periodic reviews by JECFA over the years indicate the progression of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models, which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years, as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic, and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

As discussed in Appendix 11 and elsewhere, at its 51st meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0-2 mg per kg bw per day with an adequate margin of safety (Toyoda et al., 1997). The Committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies validated the Committee conclusion that the *in vitro* mutagenic activity of steviol did not present a risk of carcinogenic effects *in vivo* and, therefore, all common steviol glycosides that likely share the same basic metabolic and excretory pathway and that use high purity preparations of various steviol glycosides, are safe as a sugar substitute. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0-4 mg per kg bw per day (based on steviol equivalents).

Recently, JECFA published a safety evaluation of a number of food additives, including steviol glycosides. The JECFA committee reviewed information supporting the safety of a *Yarrowia lipolytica* fermentation-produced rebaudioside A, which included a 90-day rat toxicity study and two *in vitro* genotoxicity studies, as well as *in vitro* colonic microflorae hydrolysis studies in several

[7] It has also been reported that steviol glycosides may have pharmacological properties, which can be used to treat certain disease conditions such as hypertension and type 2 diabetes. Chatsudthipong and Muanprasat (2009), as well as others, have published reviews where they note that such therapeutic applications have not been firmly established as being due to steviol glycosides. The reviewers point out that the effects occur at higher doses than would be used for sweetening purposes. Furthermore, many effects noted in older studies may have been due to impurities in preparations that do not meet the contemporary purity specifications established by JECFA for use as a sweetener. If oral doses of steviol glycosides impart pharmacological effects, such effects would undoubtedly occur due to actions of the principal metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated. For a more comprehensive discussion of this subject, see Section 7 of Appendix 9.

steviol glycosides, toxicokinetic studies of stevioside in humans and rats, and literature published since the 69th meeting.

The Committee noted that the most recent short-term toxicity studies were consistent with those reviewed at or prior to the 69th meeting, and that the new toxicokinetic study in humans did not have a large enough subject pool to provide reliable toxicokinetic estimates to derive an update ADI for steviol glycosides. The Committee confirmed the current ADI of 0-4 mg per kg bw steviol. In addition, the Committee prepared new "tentative" specifications for steviol glycosides, which was expanded to include "any mixture of steviol glycosides compounds derived from *S. rebaudiana* Bertoni" while retaining the requirement that the total percentage of steviol glycosides is ≥ 95% (WHO, 2017).

GLG critically reviewed the JECFA assessments and agrees with the calculation of the ADI for steviol glycosides.

Several published and unpublished studies (summarized in Appendix 12) on purified preparations of rebaudioside A showed an absence of toxicological effects in rats (Curry and Roberts, 2008; Nikiforov and Eapen, 2008) and dogs (Eapen, 2008) in subchronic studies, and an absence of reproductive (Curry and Roberts, 2008; Sloter, 2008a) and developmental effects (Sloter, 2008b) in rats. Most notably, pharmacokinetic studies in rats (Roberts and Renwick, 2008) and humans (Wheeler et al., 2008) on purified rebaudioside A follow the same pathway of being degraded to steviol by intestinal bacteria with subsequent rapid glucosylation and elimination in urine and feces.

GLG concludes that these studies on rebaudioside A strengthen the argument that all steviol glycosides that follow the same metabolic pathway are safe at the JECFA established ADI.

GLG has also reviewed the findings from human clinical studies, noting that ---with regard to the clinical effects reported in humans--- in order to corroborate the observations in these studies that these effects of steviol glycosides only occur in patients with either elevated blood glucose or blood pressure (or both). JECFA called for studies in individuals that are neither hypertensive nor diabetic (WHO, 2006). The supplemental data presented to JECFA and also published by Barriocanal et al. (2008) demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg per kg bw per day in normal individuals, or approximately slightly more than 4 mg per kg bw on the basis of steviol equivalents (Barriocanal et al., 2008). Clinical studies on purified rebaudioside A showed an absence of effects on blood pressure (Maki et al., 2008a) and blood glucose levels (Maki et al., 2008b) at doses slightly higher than the exposures expected in food. GLG concludes that there will be no effects on blood pressure and glucose metabolism in humans at the doses of steviol glycosides expected from its use in food as a non-nutritive sweetener.

Two previously published studies summarized in Appendix 9 raised a potential concern regarding the toxicological effects of steviol glycosides. In one study, DNA damage was seen in a variety of organs as assessed by Comet assay in rats given drinking water containing 4 mg per mL steviol glycosides for up to 45 days (Nunes et al., 2007). Several experts in the field have since

questioned the methodology used in this study (Geuns, 2007; Williams, 2007; Brusick, 2008). GLG has reviewed the cited publications, along with the responses made by the authors (Nunes et al., 2007b; Nunes et al., 2007c), and concurs with the challenges to the methodology utilized by Nunes et al. (2007), thereby discounting the validity and importance of this study.

In another study with stevioside in rats, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased at doses as low as 15 mg per kg bw (Awney et al., 2011). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells. This enzyme was not measured in any previous toxicology studies on steviol glycosides, nor has it been adequately vetted for application in toxicological studies. Critical reviews of this study by Carakostas (2012) and (Waddell, 2011) revealed a poor study design that included: insufficient numbers of animals; group-housing with the potential for stress-related changes; unreliable access to steviol *via* drinking water resulting in suspect dosing calculations in group-housed cages; no indication of fasting prior to blood collection (which affects many chemistry and hematological values); no urine collection; and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the report did not adequately describe mean or individual organ weight data, and it lacked comparison of study findings against laboratory historical control data.

Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern has been expressed in two relatively recent publications (Brahmachari et al., 2011; Tandel, 2011) in which the authors concluded that additional testing is necessary to adequately address the genotoxicity profile (Urban et al., 2013). The review aimed to address this matter by evaluating the specific genotoxicity studies of concern, while evaluating the adequacy of the database that includes more recent genotoxicity data not noted in these publications. The results of this literature review showed that the current database of *in vitro* and *in vivo* studies for steviol glycosides is robust and does not indicate that either stevioside or rebaudioside A is genotoxic. This finding, combined with a paucity of evidence for neoplasm development in rat bioassays, establishes the safety of all steviol glycosides with respect to their genotoxic/carcinogenic potential.

In addition, a recent paper by Shannon et al. (2016) raises a possible concern of endocrine disruption by steviol. GLG reviewed the publication and notes that the effects on progesterone production and on the action of progesterone (both antagonistic and agonistic) were observed *in vitro* in sperm cells. GLG concludes that it is difficult to translate *in vitro* concentrations to local concentrations *in vivo* at receptors and that no adverse effects were observed in well-conducted reproductive toxicology studies. Therefore, this study does not alter GLG's opinion that steviol glycosides preparations are generally recognized as safe. A summary of this study is provided in Appendix 13.

Philippaert et al. (2017) demonstrated that stevioside, rebaudioside A, and steviol potentiate the activity of transient receptor potential cation channel subfamily melastatin member 5 (TRPM5), a Ca^{2+}-activated cation channel that is expressed in type II taste receptor cells and pancreatic β-

cells. The authors found that the steviol glycosides increased the perception of bitter, sweet, and umami tastes and also enhanced glucose-induced insulin secretion in a TRPM5-dependent manner. Furthermore, *in vivo* studies indicated that daily consumption of stevioside prevents high-fat-induced diabetic hyperglycemia development in wild-type mice. No adverse events or animal deaths were discussed.

A commercially available steviol glycoside extract (>99%, composition and brand unknown) was used to investigate genotoxicity in human peripheral blood lymphocytes. Uçar et al. (2017) observed no significant differences in chromosomal aberration induction or micronuclei between the control and treatment groups at 24 and 48 hr. These data support previous findings that steviol glycosides are not genotoxic.

Panagiotou et al. (2018) observed that steviol and steviol glycosides exert glucocorticoid receptor-mediated effects in human leukemic T-cells (Jurkat cells) but not in normal human peripheral blood mononuclear cells, which they concluded was due to a cell-type specific manner of glucocorticoid receptor-modulation.

Thøgersen et al. (2018) investigated the effect of rebaudioside A, stevioside, and steviol on porcine cytochrome p450 (CYP) expression and activity to assess their potential food-drug interactions in the IPEC-J2 cell line, which is a non-transformed cell line derived from intestinal porcine epithelial cells and in primary hepatocytes. The authors reported that there were no changes in CYP messenger ribonucleic acid (mRNA) expression following treatment of IPEC-J2 cells with rebaudioside A, stevioside, and steviol compared with control. Treatment of primary hepatocytes resulted in increases in CYP329 mRNA at low concentrations of rebaudioside A and steviol, and at all concentrations of stevioside. The authors reported that while treatment with the steviol glycosides tested over 24 hours resulted in minor increases in CYP3A29 mRNA expression (< 2.0-fold), “no direct effect on CYP activity” was observed. The authors concluded that rebaudioside A, stevioside, and steviol are unlikely to cause a food-drug interaction but noted that the study could not predict long term effects and effects *in vivo*.

A recently published study addressed the genotoxic activity of stevia (Svetia™, purity not reported[8]). Human lymphocytes were treated with 5% and 0.5% Svetia™ for 2 hours. No statistically significant difference in genetic damage was observed in the 0.5% treatment concentration compared to the negative control, while the 5% treatment concentration resulted in a statistically significant difference (P<0.0001) compared to the control, with a decrease in migration average. The authors described the effect as being beneficial. Human lymphocytes treated with 10% Svetia™ demonstrated significant (P<0.0001) genotoxic activity compared to the control; however, at treatment concentrations of 0.05%, 0.5%, and 5% Svetia™, a significant (P<0.0001) decrease in average migration of DNA was observed compared to the control. The authors conclude that these results demonstrate the absence of genotoxicity at concentrations up to 5%

[8] While the purity of the material used for the study was not reported by Silva et al. (2018), a search of the manufacturer’s website (www.svetia.us) indicates that the trademarked material is a blend of cane sugar and 97% pure Reb A.

Svetia™ (Silva et al., 2018). It should be noted that these observations are inconsistent with data reported by Nunes et al. (2007); however, as discussed above, the validity and importance of the Nunes et al. study has been discounted given the questions surrounding the methodology.

Chen et al. (2018) investigated the kinetics of steviol glycoside glucuronidation in human liver microsomes and a recombinant human uridine 5'-diphospho-(UDP) glucuronosyltransferase, UGT2B7. Steviol glucuronide was the sole product of steviol glycoside glucuronidation and steviol showed strong substrate inhibition of HLM and UGT2B7. The authors also reported that stevioside and rebaudioside A did not have a notable effect on the glucuronidation of steviol. Based on the predicted hepatic clearance of steviol, the authors inferred that steviol exhibits high clearance.

As detailed in GRN 473, PureCircle Ltd. studied the metabolism of rebaudioside X (i.e., Reb M) by *in vitro* methods (PureCircle, 2013b) similar to those used in previous studies with enzyme treated stevia extract (Koyama et al., 2003b; NOWFoods, 2010) and Rebaudioside D (PureCircle, 2013a; Nikiforov et al., 2013). Rebaudioside X (Reb M) was incubated with pooled fecal homogenates over the course of 24 hours at 37°C under anaerobic conditions. After 16 hours, the rebaudioside X (Reb M) was completely hydrolyzed to steviol. In a parallel study, rebaudioside A was also completely converted to steviol after 16 hours of incubation. Reb A was metabolized more quickly than Reb X (Reb M), and the observation was attributed to the two additional glucose moieties being present in Reb X (Reb M) (PureCircle, 2013b).

The results of this study were recently published comparing anaerobic *in vitro* metabolism of rebaudiosides A, B, D, and M (Purkayastha et al., 2014). In all cases, the rebaudiosides were hydrolyzed to steviol within 24 hours with the majority of metabolism occurring within the first 8 hours. Metabolism of rebaudiosides took longer at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL). There were no marked differences in rate or extent of hydrolysis observed between male and female fecal homogenates or the individual rebaudiosides (Purkayastha et al., 2014). Results from this study corroborate the presumption of safety of rebaudioside M, given that it is observed to have a similar metabolism to that of Reb A.

GLG agrees with the safety conclusions of the 56 GRAS Expert Panels in the notifications for steviol glycosides previously submitted to FDA that resulted in "no questions" responses from FDA (as summarized in Appendix 10), JECFA (WHO, 2006; WHO, 2008), and Renwick (2008) that a sufficient number of good quality health and safety studies exist to support the determination that purified preparations of steviol glycosides, when added to food at levels up to full replacement of sucrose on a sweetness equivalency basis, meet FDA’s definition of safe.

GLG concludes that it is reasonable to apply the JECFA ADI of 4 mg per kg bw per day for steviol glycosides (expressed on a steviol basis) to GLG’s Festeviol™ RM 95. Therefore, with the steviol equivalence values shown in Table 3, GLG concludes that, for the general population, the estimated maximum daily intake of GLG’s Festeviol™ RM 95 is 4.95 mg per kg bw or 1.22 mg per kg expressed as steviol equivalents. Based upon these calculations, the intake of GLG’s

Festeviol™ RM 95 safely aligns with the 4 mg per kg bw per day ADI expressed as steviol equivalents as determined by JECFA.

GLG's Festeviol™ RM 95 preparation contains not less than 95% rebaudioside M. Given the structural similarities with rebaudioside A, stevioside, and other steviol glycosides, and considering analogous metabolic pathways for all these substances, the safety data on stevia and its other components have a direct bearing on the present safety assessment for Festeviol™ RM 95. This is further supported by over a decade and a half of scientific studies on the safety of these substances, along with the fact that the major regulatory bodies view the results of toxicology studies on either stevioside or rebaudioside A as applicable to the safety assessment of all known steviol glycosides, since all are metabolized and excreted by similar pathways, with steviol being the common metabolite for each. The foundational safety of Reb A, other steviol glycosides and steviol has been summarized, with key studies detailed in Appendix 9, Appendix 11, Appendix 12, and Appendix 13.

In addition, GLG affirms that its Festeviol™ RM 95 preparation is manufactured under CGMP conditions with raw materials and processing aids that meet the appropriate food grade regulations. GLG has established sufficient rigorous product specifications based upon FCC and JECFA monographs---which are consistent with other steviol glycosides on the market---and has demonstrated batch-to-batch consistency against these specifications.

Furthermore, GLG has reviewed this safety information and has concluded that Festeviol™ RM 95 is generally recognized as safe for the proposed uses.

A. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."[9]

Amplification is provided in that the conclusion of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

> "…General recognition of safety requires common knowledge, throughout the expert scientific community knowledgeable about the safety of substances directly

[9] See 21 CFR 170.3 (e)(i) and 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 9/8/18).

> or indirectly added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use."
>
> "'Common knowledge' can be based on either "scientific procedures" or on experience based on common use of a substance in food prior to January 1, 1958." [10]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[11]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive. General recognition of safety through scientific procedures shall be based upon the application of generally available and accepted scientific data, information, or methods, which ordinarily are published, as well as the application of scientific principles, and may be corroborated by the application of unpublished scientific data, information, or methods.

The apparent imprecision of the terms "appreciable," "at the time," and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

As noted below, this safety assessment to ascertain GRAS status for high purity steviol glycosides for the specified food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

B. Expert Panel Findings on Safety of GLG's Festeviol™ RM 95

An evaluation of the safety and GRAS status of the intended use of GLG's Festeviol™ RM 95 preparation has been conducted by an Expert Panel convened by GRAS Associates; the Panel consisted of Doug Archer, Ph.D.; Kara Lewis, Ph.D.; and Katrina Emmel, Ph.D., as Panel Chair. The Expert Panel reviewed GLG's dossier as well as other publicly available information. The

[10] See 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 9/8/18).
[11] See Footnote 1.

individuals who served as Expert Panelists are qualified to evaluate the safety of foods and food ingredients by merit of scientific training and experience.

The GRAS Expert Panel report is provided in Appendix 14.

C. Common Knowledge Elements for GRAS Conclusions

The first common knowledge element for a GRAS conclusion requires that data and information relied upon to establish safety be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The second common knowledge element for a GRAS conclusion requires that consensus exist within the broader scientific community.

1. Public Availability of Scientific Information

The majority of the studies reviewed on steviol glycosides and steviol have been published in the scientific literature as summarized in Appendix 9, Appendix 11, and Appendix 13. Most of the literature relied upon by JECFA has also been published---most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the gastrointestinal (GI) tract, *per se*; they are converted to steviol by bacteria normally present in the large intestine, and the steviol is absorbed but rapidly metabolized and excreted (Gardana et al., 2003; Koyama et al., 2003b). The action of bacteria in the large intestine is directly supported by the published study that showed that steviol glycosides can be converted to steviol in the large intestine by normal anaerobic GI flora as demonstrated by an *in vitro* study in fecal homogenates (Koyama et al., 2003b; Renwick and Tarka, 2008).

The ADI for steviol glycosides has been set largely based on a published chronic study in rats (Toyoda et al., 1997) and several published clinical studies that report no pharmacological effects in humans at doses several fold higher than the ADI (Barriocanal et al., 2006; Barriocanal et al., 2008; Wheeler et al., 2008). As mentioned above, Roberts et al. (2016) noted that the ADI could be higher using a chemical-specific adjustment factor (CSAF), as defined by the World Health Organization (WHO) in 2005, determined by comparative studies in rats and humans, which they conclude can justify an ADI value of 6-16 mg per kg bw per day for steviol glycosides.

The toxicity of the metabolite, steviol, has been well reviewed in the published literature (Geuns et al., 2003a; WHO, 2006; Urban et al., 2013).

Studies regarding rebaudioside M isolation, structural determination, and metabolism have been published (Chaturvedula et al., 2013; Prakash et al., 2014; Purkayastha et al., 2014) in the

literature. In addition, there is a large, publicly available, collection of GRNs regarding steviol glycosides on FDA's website.

2. Scientific Consensus

The second common knowledge element for a GRAS conclusion requires that there be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

A number of well-respected regulatory agencies, including JECFA, EFSA, FSANZ, the Switzerland Office of Public Health, and Health Canada, as well as numerous well-respected individual scientists, have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (FAO, 2010; EFSA, 2010; FSANZ, 2008; Health, 2008; Health Canada, 2012; Xili et al., 1992; Toyoda et al., 1997; Geuns et al., 2003a; Williams, 2007). Since December 2008, over fifty-five GRAS notifications have been submitted to FDA for highly purified stevia-derived sweetener products, and FDA's detailed reviews have consistently yielded "no questions" letters.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of rebaudioside M when of sufficiently high purity. The central role of conversion to steviol and subsequent elimination with these naturally occurring steviol glycosides extends to the manner in which the various steviol glycosides molecules are metabolized and eliminated from the body. While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, GLG believes that a wide consensus does exist in the scientific community to support a GRAS conclusion as evidenced by several publications (Carakostas, 2012; Geuns, 2007; Urban et al., 2013; Waddell, 2011; Williams, 2007; Brusick, 2008) that refute safety concerns expressed by a minority of scientists. Roberts et al. (2016) suggests that the ADI could be higher than has been previously accepted by the scientific community.

D. Conclusion

In consideration of the aggregate safety information available on naturally occurring steviol glycosides, GLG concludes that Festeviol™ RM 95, as defined in the subject notification, is safe for use as a general-purpose non-nutritive sweetener in foods other than infant formulas and meat and poultry products. The JECFA ADI for steviol glycosides of 4 mg per kg bw per day (as steviol equivalents) can be applied to GLG's Festeviol™ RM 95 preparation. Based on published dietary exposure data for other approved sweeteners and adjusting for relative sweetness intensity, intake was estimated for healthy non-diabetic children and adults, and diabetic children and adults with the following findings.

The worst-case estimated intakes of GLG's Festeviol™ RM 95 for several population groups summarized in Part 3.A. are no greater than 1.22 mg per kg steviol equivalents per bw per day, which is below the ADI of 4 mg per kg bw expressed as steviol equivalents as established by

JECFA. The dietary levels from anticipated food consumption are not likely to exceed the ADI when Festeviol™ RM 95 is used as a general non-nutritive sweetener.

Accordingly, Festeviol™ RM 95 as produced by GLG and declared within the subject notification meets FDA's definition of safety in that there is "reasonable certainty of no harm under the intended conditions of use" as described herein and, therefore, is generally recognized as safe (GRAS).

PART 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS NOTICE

A. List of Acronyms and References

1. List of Acronyms

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the plasma-concentration time curve
AVA	Agri-food and Veterinary Authority of Singapore
BP	Blood pressure
bw	Body Weight
C	Celsius
CFU	Colony Forming Unit
CGMP	Current Good Manufacturing Practice
C_{max}	Maximum serum concentration
CSAF	Chemical-Specific Adjustment Factor
CYP	Cytochrome P450
DBP	Diastolic blood pressure
dL	Deciliter
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
EU	European Union
FCC	Food Chemicals Codex
FD&C	Federal Food Drug and Cosmetics Act
FEMA	Flavor Extract Manufacturers Association
FOIA	Freedom of Information Act
FSANZ	Food Standards Australia New Zealand
FSSAI	Food Safety and Standards Authority of India
GA	GRAS Associates
GEMS	Global Environment Monitoring System
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
GPT	Glutamic-pyruvate transaminase
GRAS	Generally Recognized as Safe

GRN	GRAS Notification
HbA1c	Glycated hemoglobin
HDL	High-density lipoprotein
HPLC	High-Performance Liquid Chromatography
HR	Heart rate
hr	Hour
IADSA	International Alliance of Dietary/Food Supplement Associations
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	Kilogram
LD50	Median lethal dose
LDL	Low-density lipoprotein
MAP	Mean arterial pressure
mg	Milligram
mL	Milliliter
mm Hg	Millimeters mercury
MPL	Maximum permitted level
MPN	Most probable number
mRNA	Messenger ribonucleic acid
ng	Nanogram
NHANES	National Health and Nutrition Examination Surveys
NHPs	Natural Health Products
NIH	National Institutes of Health
NMT	Not more than
NOAEL	No observed adverse effect level
NOEL	No observed effect level
PCV%	Packed cell volume
Ph.D.	Doctor of Philosophy
PND	Post natal day
ppm	Parts per million
RBC	Red blood cell
SBP	Systolic blood pressure
SCF	European Commission's Scientific Committee on Food
$T_{1/2}$	Half-life
TAC	Total antioxidant capacity
tds	Total dissolved solids
TFC	Total flavonoid content
TK	Toxicokinetic
T_{max}	Time to maximum plasma concentration
TPC	Total phenolic content
TRAP	Tartrate-resistant alkaline phosphatase
TRPM5	Transient receptor potential cation channel subfamily melastatin member 5
UDP	Uridine diphosphate
ug	Microgram
US	United States
uU	Microunits
VLDL	Very low-density lipoprotein
WBC	White blood cell
WHO	World Health Organization
WHO/JECFA	World Health Organizaiton/Joint FAO/WHO Expert Committee on Food Additives

2. References

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B. Appendices

Appendix 1 Specifications and Certificates of Analyses for Raw Materials and Production Processing Aids

Appendix 1.1 Stevia Extract

Product Specification Sheet

File No.: GLG-QA-STD-015
Reviewed by: Zhang Lei, QA Manager
Approved by: Kevin Li, Vice President
Date: 04/20/2016

Product Name: Rebpure™ RA97

Product Description:

Rebpure™ RA97 is a highly purified extract containing rebaudioside A (MW: 967.03) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages. Rebpure™ RA97 meets JECFA requirements completely.

Brand: Rebpure™

Shelf Life: 2 years

Physical and Organoleptic Standards

CHARACTERISTIC	SPECIFICATION	METHOD
Appearance	White/off-white hygroscopic powder	Organoleptic AS IS
Flavor	Sweet	Organoleptic AS IS
Aroma	Sweet	Organoleptic AS IS
Solubility	Freely soluble in water, colorless and clear	Ro Tap 25g for 5 min

Specification

CHARACTERISTIC	SPECIFICATION	METHOD
Rebaudioside A (wt/wt)	≥ 97% (on dry basis)	JECFA 2010
Total Steviol Glycosides (wt/wt)	≥ 97% (on dry basis)	JECFA 2010
Total Heavy Metals	≤ 10 ppm	USP<231>
-Arsenic (as As)	≤ 1.0 ppm	JECFA Vol.4
-Lead (as Pb)	≤ 1.0 ppm	JECFA Vol.4
Loss on Drying	≤ 4.0 %	JECFA Vol.4
PH	4.5-7.0	JECFA Vol.4
Residue on Ignition	≤ 1.0%	JECFA Vol.4
Residual solvents, total	≤ 5200 ppm	JECFA Vol.4
-Ethanol	≤ 5000 ppm	JECFA Vol.4
-Methanol	≤ 200 ppm	JECFA Vol.4

Microbiological Standards:

CHARACTERISTIC	LIMIT	UNITS	METHOD
Total Plate Count	< 1000	cfu/g	FDA-BAM chapter 3
Yeast & Mold	< 100	cfu/g	FDA-BAM chapter 18
E.coli	< 3	MPN/g	FDA-BAM chapter 4
Staphylococcus aureus	< 10	cfu/g	FDA-BAM chapter 12
Salmonella (/25g)	Negative	(/25g)	FDA-BAM chapter 5

Suite100 - 10271 Shellbridge Way * Richmond, B.C., Canada * V6X 2W8
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Page 1 of 2

Product Specification Sheet (Continued)

GLG
LEADING LIFE TECHNOLOGIES
BETTER STEVIA, MONK FRUIT AND MORE

File No.: GLG-QA-STD-015
Reviewed by: Zhang Lei, QA Manager
Approved by: Kevin Li, Vice President
Date: 04/20/2016

Storage and Handling

Transport of the product shall be under such conditions that will prevent contamination. The product shall be stored in a sealed container in a cool, dry place.

Packaging

The product shall be shipped in packaging that is suitable for inland and ocean transportation. It shall be contained in a suitable inner bag (e.g. plastic). The inner bag shall be contained in an appropriate outer container (e.g. suitable cardboard box) and the outer container should have a conspicuous label on the side of the outer container. The outer container label shall be legible, indelible and permanent and indicate the proper name of the product, the lot number, purchaser name and country of origin.

Product Guarantee

This product was produced in a plant that conforms to Good Manufacturing Practices and meets state and federal regulations. This product has critical control points to protect against the inclusion of metal or other extraneous material in the product. GLG Life Tech Corporation warrants that the lead contained in the product occurs naturally and is ≤1ppm. The product meets the requirements listed in this specification sheet unless otherwise stated by GLG Life Tech Corporation. A certificate of analysis is supplied with each lot of Rebpure™ RA97 and shall include the name and location of the production facility.

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Appendix 1.2 Sucrose

Product Specification Sheet

File No.: GLG-QA-STD-150
Reviewed by: Zhang Lei, QA Manager
Approved by: Kevin Li, Vice President
Date: 04/20/2018

Product Name: Sucrose

Product Description:

Sucrose is extracted, and refined, from either sugar cane or sugar beet.

Shelf Life: 2 years

Physical and Organoleptic Standards

CHARACTERISTIC	SPECIFICATION	METHOD
Appearance	White crystal	Organoleptic AS IS
Flavor	Sweet	Organoleptic AS IS
Aroma	Sweet	Organoleptic AS IS
Particle size	40 – 100 mesh	Ro Tap 25g for 5 minutes

Specification

CHARACTERISTIC	SPECIFICATION	METHOD
Sucrose, g/100g	≥99.7	GB/T 35887-2018
Reducing Sugar, g/100g	≤0.04	GB/T 35887-2018
Conductometric Ash, g/100g	≤0.04	GB/T 35887-2018
Loss on Drying, g/100g	≤ 0.06	GB/T 35887-2018
Color Value, IU	≤ 60	GB/T 35887-2018
Turbidity, MAU	≤ 80	GB/T 35887-2018
Impurity insoluble in water, mg/kg	≤ 20	GB/T 35887-2018
Heavy Metals -Arsenic (as As) -Lead (as Pb)	 ≤ 0.5ppm ≤ 0.5ppm	 AAS AAS

Microbiological Standards:

CHARACTERISTIC	LMIT	UNITS	METHOD
Acarid	Negative	Negative	GB 13104-2014
Total Plate Count	<1000	CFU/g	FDA-BAM chapter 3
Yeast & Mold	< 100	CFU/g	FDA-BAM chapter 18
E.coli	<3	MPN/g	FDA-BAM chapter 4
Staphylococcus aureus	<10	CFU/g	FDA-BAM chapter 12
Salmonella (/25g)	Negative	Negative	FDA-BAM chapter 5

Page 1 of 2

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Product Specification Sheet (Continued)

GLG
LEADING LIFE TECHNOLOGIES
BETTER STEVIA, MONK FRUIT AND MORE

File No.: GLG-QA-STD-150
Reviewed by: Zhang Lei, QA Manager
Approved by: Kevin Li, Vice President
Date: 04/20/2018

Storage and Handling

Transport of the product shall be under such conditions that will prevent contamination. The product shall be stored in a sealed container in a cool, dry place.

Packaging

The product shall be shipped in packaging that is suitable for inland and ocean transportation. It shall be contained in a suitable inner bag (e.g. plastic). The inner bag shall be contained in an appropriate outer container (e.g. suitable cardboard box) and the outer container should have a conspicuous label on the side of the outer container. The outer container label shall be legible, indelible and permanent and indicate the proper name of the product, the lot number, purchaser name and country of origin.

Product Guarantee

This product was produced in a plant that conforms to Good Manufacturing Practices and meets state and federal regulations. This product has critical control points to protect against the inclusion of metal or other extraneous material in the product. GLG Life Tech Corporation warrants that the lead contained in the product occurs naturally and is ≤1ppm. The product meets the requirements listed in this specification sheet unless otherwise stated by GLG Life Tech Corporation. A certificate of analysis is supplied with each lot of Sucrose and shall include the name and location of the production facility.

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Appendix 1.3 Ethanol

Anhui anle food co. ltd NO.ATC/19-001R/1.1

Certificate of Analysis

Product name	Edible Alcohol			Sampling date	2018.05.18	
Inspection basis	AT-JYZ-31/1.1			Batch number	[redacted]	
Test results						
Inspection item	UNIT	Superfine alcohol	Optimal levels of alcohol	Ordinary alcohol	Execution standard	Result
Appearance	/	A colorless transparent liquid			GB 31640-2016	Qualified
Smell	/	Has inherent ethanol aroma, no other odors			GB 31640-2016	Qualified
Taste	/	Pure, slightly sweet and odorless			GB 31640-2016	Qualified
Chroma	No≤	10			GB 10343-2008	5
Alcoholic strength	%vol≥	96	95.5	95	GB 31640-2016	95.6
Sulfuric acid test	No≤	10		60	GB 10343-2008	8
Oxidation time	min≥	40	30	20	GB 10343-2008	35
Aldehyde (in the form of acetaldehyde)	mg/l≤	1	2	30	GB 31640-2016	1.4
Methanol	mg/l≤	2	50	150	GB 31640-2016	31
Normal Propyl Alcohol	mg/l≤	2	15	100	GB 10343-2008	8.6
Isobutyl alcohol+Isoamyl alcohol	mg/l≤	1	2	30	GB 10343-2008	ND
Acid (in the form of acetic acid)	mg/l≤	7	10	20	GB 10343-2008	7.7
Lead(Pb)	Mg/kg≤	1.0			GB 31640-2016	0.2
Detection conclusion	Optimal levels of alcohol					

Analyzed by: [redacted] Approved by [redacted] Date: 2018.5.18.

Company address: no. 7, jintai fifth road, wuzhou economic development zone Mail editor: 234200
Tel: 0557-2282621 Fax: 0557-2282626 Mail box: anle@anlegroup.com Web site: www.anlegroup.com

Appendix 1.4 Macro Porous Adsorbent Resin

Driving the innovation
SUNRESIN
Sunresin--China Biggest Special Resin Manufacturer

Certificate of Analysis

Product	LX-T28	Product grade	superior
Batch No.	[redacted]	Produce Date	2018.11.19
		Analysis Date	2018.11.20
Standard No.	Q/LX 008-2017	Appearance	White opaque spherical particles

No.	Items	Specifications	Data From Analysis
1	Particl size(0.315-1.25 mm) (%)	≥95.0	98.50
2	Moisture（%）	55-65	57.01
3	Shipping weight (g/ml)	0.62-0.67	0.66
4	Density (g/ml)	1.00-1.10	1.03
5	Surface area (m^2/g)	≥500	521.28
6			
7			
8			
Issued by	[redacted]	Confirmed by	[redacted]

SUNRESIN NEW MATERIALS CO.LTD. XI'AN Q.C.Dept

Sunresin New Materials Co. Ltd., Xi'an

Certificate Of Compliance
201410-201509

This is certified that:

At The Address Stated Below Has Completed U.S. FOOD And DRUG ADMINISTRATION Food Facility Registration And Test Through MANTONG.

CHUZHOU RUNHAI STEVIA HIGH TECH CO., LTD.
INDUSTRIAL PARK, MINGGUANG CITY, ANHUI PROVINCE,CHINA

Product Description	1、Macroporous Adsorption Resin Style/ Item No.: D101 Report No.:DC176058 2、Macroporous Week Alkaline Styrene Type Anion Exchange Resin Style/ Item No.: D301R Report No.:DC176059
Test Requested	FDA 21 CFR 173.25
Supplier:	Tianjin Nankai HECHENG S&T CO., Ltd
Performing Date	2014-10-11 to 2014-10-21
Test Laboratory	STC
Registration Number	18702249954

MTG FDA REGISTER CENTER CORPORATE SEAL CALIFORNIA

[redacted]
Executive Director
Date: 10-23-2014

MTG STANDARDS TESTING & CERTIFICATION CENTER www.fdacn.org

STC Test Report

Date : 2014-10-21
No. : DC176058

Applicant(Code: CZR001) : CHUZHOU RUNHAI STEVIA HIGH TECH CO., LTD. INDUSTRIAL PARK, MINGGUANG CITY, ANHUI PROVINCE, CHINA

Description of Sample(s) : Sample(s) received is/ are stated to be:
Name: Macroporous Adsorption Resin
Style/ Item No.: D101
Supplier: Tianjin Nankai HECHENG S&T CO., Ltd.

Date Sample(s) Received : 2014-10-11

Date Tested : 2014-10-11 to 2014-10-21

Result Summary:

No.	Test Requested	Conclusion	Remark
1	Ion-exchange resins – FDA 21 CFR 173.25 - Organic extractives in distilled water - Organic extractives in 15%(v/v) alcohol	PASS	-

Peng Wen Qi, Boly
Authorized Signatory
Chemical, Food and Pharmaceutical Department
For and on behalf of
STC (Dongguan) Company Ltd.

STC (Dongguan) Company Limited
[illegible]

STC Test Report

Date : 2014-10-21 **Page 2 of 3**
No. : DC176058 **(Duplicate)**

RESULT(S):

ION-EXCHANGE RESINS – FDA 21 CFR 173.25
Method Used: Ref. 21 CFR 173.25
- Organic extractives in distilled water
- Organic extractives in 15%(v/v) alcohol

Sample Identity	Colour / Component	Style
1	White resin (Macroporous Adsorption Resin)	-

Test Item(s)	Unit	Result(s)	Limit
		1	
- Organic extractives in distilled water	ppm	0.7	1
- Organic extractives in 15%(v/v) alcohol	ppm	0.3	1
Conclusion	-	Pass	-

Note(s): - ND = Not Detected
- Method detected limit: 0.1 ppm
- ppm = part(s) per million = mg/kg = milligram per kilogram

Remark(s): - As per confirmation by client, the submitted sample(s) was the materials of (a)(1) Sulfonated copolymer of styrene and divinylbenzene.

STC (Dongguan) Company Limited
[illegible]

STC Test Report

Date : 2014-10-21 **Page 3 of 3**
No. : DC176058 **(Duplicate)**

PHOTO(S):

***** END OF TEST REPORT *****

STC (Dongguan) Company Limited
68 Fumin Hao Road, Dalang, Dongguan, China. (Zip Code : 523 770)
Tel : (86 769) 8111 8888 Fax : (86 769) 8111 8222 E-mail : dgstc@dgstc.org Homepage : www.dgstc.org

STC Test Report

Date : 2014-10-21
No. : DC176059

Applicant(Code: CZR001) : CHUZHOU RUNHAI STEVIA HIGH TECH CO., LTD. INDUSTRIAL PARK, MINGGUANG CITY, ANHUI PROVINCE, CHINA

Description of Sample(s) : Sample(s) received is/ are stated to be:
Name: Macroporous Week Alkaline Styrene Type Anion Exchange Resin
Style/ Item No.: D301R
Supplier: Tianjin Nankai HECHENG S&T CO., Ltd.

Date Sample(s) Received : 2014-10-11

Date Tested : 2014-10-11 to 2014-10-21

Result Summary:

No.	Test Requested	Conclusion	Remark
1	Ion-exchange resins – FDA 21 CFR 173.25 - Organic extractives in distilled water - Organic extractives in 15%(v/v) alcohol	PASS	-

Peng Wen Qi, Boly
Authorized Signatory
Chemical, Food and Pharmaceutical Department
For and on behalf of
STC (Dongguan) Company Ltd.

STC (Dongguan) Company Limited
69 Fumin Nan Road, Daning, Dongguan, China. (Zip Code: 523 770)
Tel: (86 769) 8111 3888 Fax: (86 769) 8111 3222 E-mail: dgstc@sgsgtc.org Homepage: www.dgstc.org

STC Test Report

Date : 2014-10-21
No. : DC176059

RESULT(S):

ION-EXCHANGE RESINS – FDA 21 CFR 173.25
Method Used: Ref. 21 CFR 173.25
- Organic extractives in distilled water
- Organic extractives in 15%(v/v) alcohol

Sample Identity	Colour / Component	Style
1	Beige resin (Macroporous Week Alkaline Styrene Type Anion Exchange Resin)	-

Test Item(s)	Unit	Result(s)	Limit
		1	
- Organic extractives in distilled water	ppm	0.5	1
- Organic extractives in 15%(v/v) alcohol	ppm	0.7	1
Conclusion	-	Pass	-

Note(s): - ND = Not Detected
- Method detected limit: 0.1 ppm
- ppm = part(s) per million = mg/kg = milligram per kilogram

Remark(s): - As per confirmation by client, the submitted sample(s) was the materials of (a)(1) Sulfonated copolymer of styrene and divinylbenzene.

STC (Dongguan) Company Limited
[illegible]

STC Test Report

Date : 2014-10-21 **Page 3 of 3**
No. : DC176059 **(Duplicate)**

PHOTO(S):

***** END OF TEST REPORT *****

STC (Dongguan) Company Limited
68 Fumin Nan Road, Daleng, Dongguan, China. (Zip Code : 523 770)
Tel : (86 769) 8111 8888 Fax : (86 769) 8111 8222 E-mail : dgstc@dgstc.org Homepage : www.dgstc.org

Appendix 2 Certificates of Analysis for Multiple Batches of Festeviol™ RM 95

Appendix 2.1 Festeviol™ RM 95 Lot [redacted]

Certificate of Analysis

GLG

File No.: GLG-QA-COA-83
Date:04/27/2017

Product: GLG Festeviol™RM 95
LotNo. [redacted]
Manufacturing Date: March 1st ,2018
Country of Origin: CHINA
Shelf Life: 2 Years

ProductDescription: Festeviol™ Reb M 95 is a highly purified extract produced through bioconversion of high purity Rebaudioside A. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

Distributed By:
GLG Life Tech Corporation
Suite 100 - 10271 Shellbridge Way
Richmond, B.C. V6X 2W8
Canada
Phone: 1.604.285.2602
Fax: 1.604.285.2606
Email: sales@glglifetech.com
Web: www.glglifetech.com

Manufactured By:
Qingdao Runde Biotechnology Co.,Ltd.
Lingshanwei Town, Jiaonan County,
Qingdao, Shandong, 266427 China
Phone: +86.532.83181169
Fax: +86.532.83181836

Qingdao Runde Biotechnology Co.,Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Manufacturer's FDA Registration Number: 19905279852

Date of Analysis: March 6th , 2018

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Organoleptic AS IS
Particle Size	100% pass through 80 mesh	Conform	Ro Tap
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.29%	JECFA 2017
Total Steviol Glycosides (wt/wt)	≥95.0% (on dry basis)	98.69%	JECFA 2017
Loss on Drying	≤ 6.0%	2.68%	JECFA Vol.4
pH	4.5-7.0	5.2	JECFA Vol.4
Residue on ignition	≤ 1.0%	0.05%	JECFA Vol.4
Lead (as Pb)	≤1.0 ppm	0.07 ppm	JECFA Vol.4
Arsenic (as As)	≤1.0 ppm	0.06 ppm	JECFA Vol.4
Cadmium (as Cd)	≤1.0 ppm	0.01ppm	JECFA Vol.4
Mercury (as Hg)	≤1.0 ppm	0.02ppm	JECFA Vol.4
Residual solvents -Ethanol	≤ 5000 ppm	2321.8ppm	JECFA Vol.4
-Methanol	≤ 200 ppm	38.6ppm	JECFA Vol.4
Total PlateCount	< 1000 CFU/g	< 10 cfu/g	FDA-BAM chapter 3
Yeast& Mold	<100 CFU/g	< 10 cfu/g	FDA-BAM chapter 18
E.coli	< 3 MPN/g	< 3 MPN/g	FDA-BAM chapter 4
Staphylococcus Aureus	< 10 CFU/g	< 10 CFU/g	FDA-BAM chapter 12
Salmonella(/25g)	Negative	Negative	FDA-BAM chapter 5

Conclusion	QUALIFIED

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: [redacted] **Date:** 06/03/2018
Checked by: [redacted] **Date:** 06/03/2018.
Approved by: [redacted] (Quality Manager) **Date:** 06/03/2018

Appendix 2.2 Festeviol™ RM 95 Lot [redacted]

Certificate of Analysis

GLG

File No.: GLG-QA-COA-[redacted]
Date:04/27/2017

Product:GLG Festeviol™RM 95
LotNo.: [redacted]

Manufacturing Date: March 4th ,2018
Country of Origin: CHINA
Shelf Life: 2 Years

ProductDescription: Festeviol™ Reb M 95 is a highly purified extract produced through bioconversion of high purity Rebaudioside A. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

Distributed By:
GLG Life Tech Corporation
Suite 100 - 10271 Shellbridge Way
Richmond, B.C. V6X 2W8
Canada
Phone: 1.604.285.2602
Fax: 1.604.285.2606
Email: sales@glglifetech.com
Web: www.glglifetech.com

Manufactured By:
Qingdao Runde Biotechnology Co.,Ltd.
Lingshanwei Town, Jiaonan County,
Qingdao, Shandong, 266427 China
Phone: +86.532.83181169
Fax: +86.532.83181836

Qingdao Runde Biotechnology Co.,Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Manufacturer's FDA Registration Number:19905279852

Date of Analysis: March 9th ,2018

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Organoleptic AS IS
Particle Size	100% pass through 80 mesh	Conform	Ro Tap
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.12%	JECFA 2017
Total Steviol Glycosides (wt/wt)	≥95.0% (on dry basis)	98.65%	JECFA 2017
Loss on Drying	≤ 6.0%	2.32%	JECFA Vol.4
pH	4.5-7.0	5.6	JECFA Vol.4
Residue on Ignition	≤ 1.0%	0.07%	JECFA Vol.4
Lead (as Pb)	≤1.0 ppm	0.03 ppm	JECFA Vol.4
Arsenic (as As)	≤1.0 ppm	0.02ppm	JECFA Vol.4
Cadmium (as Cd)	≤1.0 ppm	0.03ppm	JECFA Vol.4
Mercury (as Hg)	≤1.0 ppm	0.01ppm	JECFA Vol.4
Residual solvents -Ethanol	≤ 5000 ppm	1265.4ppm	JECFA Vol.4
-Methanol	≤ 200 ppm	78.9 ppm	JECFA Vol.4
Total PlateCount	< 1000 CFU/g	< 10 cfu/g	FDA-BAM chapter 3
Yeast& Mold	<100 CFU/g	< 10 cfu/g	FDA-BAM chapter 18
E.coli	< 3 MPN/g	< 3 MPN/g	FDA-BAM chapter 4
Staphylococcus Aureus	< 10 CFU/g	< 10 CFU/g	FDA-BAM chapter 12
Salmonella(/25g)	Negative	Negative	FDA-BAM chapter 5

Conclusion	QUALIFIED

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: [redacted] **Date:** 09/03/2018

Checked by: [redacted] **Date:** 09/03/2018

Approved by: [redacted] (Quality Manager) **Date:** 09/03/2018

Appendix 2.3 Festeviol™ RM 95 Lot [redacted]

Certificate of Analysis

GLG

File No.: GLG-QA-COA-83
Date:04/27/2017

Product:GLG Festeviol™RM 95
LotNo.: [redacted]

Manufacturing Date: March 6th ,2018
Country of Origin:CHINA
Shelf Life: 2 Years

ProductDescription: Festeviol™ Reb M 95 is a highly purified extract produced through bioconversion of high purity Rebaudioside A. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

Distributed By: GLG Life Tech Corporation
Suite 100 - 10271 Shellbridge Way
Richmond, B.C. V6X 2W8
Canada

Phone: 1.604.285.2602
Fax: 1.604.285.2606
Email: sales@glglifetech.com
Web: www.glglifetech.com

Manufactured By: Qingdao Runde Biotechnology Co.,Ltd.
Lingshanwei Town, Jiaonan County,
Qingdao, Shandong, 266427 China

Phone: +86.532.83181169
Fax: +86.532.83181836

Qingdao Runde Biotechnology Co.,Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Manufacturer's FDA Registration Number:19905279852

Date of Analysis: March 11th ,2018

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Organoleptic AS IS
Particle Size	100% pass through 80 mesh	Conform	Ro Tap
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.62%	JECFA 2017
Total Steviol Glycosides (wt/wt)	≥95.0% (on dry basis)	98.43%	JECFA 2017
Loss on Drying	≤ 6.0%	2.92%	JECFA Vol.4
pH	4.5-7.0	5.1	JECFA Vol.4
Residue on Ignition	≤ 1.0%	0.08%	JECFA Vol.4
Lead (as Pb)	≤1.0 ppm	0.05 ppm	JECFA Vol.4
Arsenic (as As)	≤1.0 ppm	0.03ppm	JECFA Vol.4
Cadmium (as Cd)	≤1.0 ppm	0.01ppm	JECFA Vol.4
Mercury (as Hg)	≤1.0 ppm	0.01ppm	JECFA Vol.4
Residual solvents -Ethanol	≤ 5000 ppm	1873.8ppm	JECFA Vol.4
-Methanol	≤ 200 ppm	91.4ppm	JECFA Vol.4
Total PlateCount	< 1000 CFU/g	< 10 cfu/g	FDA-BAM chapter 3
Yeast& Mold	<100 CFU/g	< 10 cfu/g	FDA-BAM chapter 18
E.coli	< 3 MPN/g	< 3 MPN/g	FDA-BAM chapter 4
Staphylococcus Aureus	< 10 CFU/g	< 10 CFU/g	FDA-BAM chapter 12
Salmonella(/25g)	Negative	Negative	FDA-BAM chapter 5

Conclusion	QUALIFIED

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: [redacted] **Date:** 11/03/2018

Checked by: [redacted] **Date:** 11/03/2018.

Approved by: [redacted] (Quality Manager) **Date:** 11/03/2018

Appendix 2.4 Festeviol™ RM 95 Lot [redacted]

Certificate of Analysis

GLG

File No.: GLG-QA-COA-83
Date:04/27/2017

Product:GLG Festeviol™RM 95
LotNo.:GLG-RM95-[redacted]

Manufacturing Date: March 8th, 2018
Country of Origin:CHINA
Shelf Life: 2 Years

ProductDescription: Festeviol™ Reb M 95 is a highly purified extract produced through bioconversion of high purity Rebaudioside A. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

Distributed By:
GLG Life Tech Corporation
Suite 100 - 10271 Shellbridge Way
Richmond, B.C. V6X 2W8
Canada

Phone: 1.604.285.2602
Fax: 1.604.285.2606
Email: sales@glglifetech.com
Web: www.glglifetech.com

Manufactured By: Qingdao Runde Biotechnology Co.,Ltd.
Lingshanwei Town, Jiaonan County,
Qingdao, Shandong, 266427 China

Phone: +86.532.83181169
Fax: +86.532.83181836

Qingdao Runde Biotechnology Co.,Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Manufacturer's FDA Registration Number:19905279852

Date of Analysis: March 13th ,2018

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Organoleptic AS IS
Particle Size	100% pass through 80 mesh	Conform	Ro Tap
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.70%	JECFA 2017
Total Steviol Glycosides (wt/wt)	≥95.0% (on dry basis)	98.90%	JECFA 2017
Loss on Drying	≤ 6.0%	2.28%	JECFA Vol.4
pH	4.5-7.0	5.7	JECFA Vol.4
Residue on Ignition	≤ 1.0%	0.07 %	JECFA Vol.4
Lead (as Pb)	≤1.0 ppm	0.04 ppm	JECFA Vol.4
Arsenic (as As)	≤1.0 ppm	0.05ppm	JECFA Vol.4
Cadmium (as Cd)	≤1.0 ppm	0.02ppm	JECFA Vol.4
Mercury (as Hg)	≤1.0 ppm	0.01ppm	JECFA Vol.4
Residual solvents -Ethanol	≤ 5000 ppm	1678.3 ppm	JECFA Vol.4
-Methanol	≤ 200 ppm	58.9 ppm	JECFA Vol.4
Total PlateCount	< 1000 CFU/g	< 10 cfu/g	FDA-BAM chapter 3
Yeast& Mold	<100 CFU/g	< 10 cfu/g	FDA-BAM chapter 18
E.coli	< 3 MPN/g	< 3 MPN/g	FDA-BAM chapter 4
Staphylococcus Aureus	< 10 CFU/g	< 10 CFU/g	FDA-BAM chapter 12
Salmonella(/25g)	Negative	Negative	FDA-BAM chapter 5

Conclusion	QUALIFIED

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: [redacted] **Date:** 13/03/2018

Checked by: [redacted] **Date:** 13/03/2018.

Approved by: [redacted] (Quality Manager) **Date:** 13/03/2018

Appendix 2.5 Festeviol™ RM 95 Lot [redacted]

Certificate of Analysis

GLG

File No.: GLG-QA-COA-83
Date:04/27/2017

Product:GLG Festeviol™RM 95
LotNo.:GLG-RM95-[redacted]

Manufacturing Date: March 10th , 2018
Country of Origin:CHINA
Shelf Life: 2 Years

ProductDescription: Festeviol™ Reb M 95 is a highly purified extract produced through bioconversion of high purity Rebaudioside A. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

Distributed By:
GLG Life Tech Corporation
Suite 100 - 10271 Shellbridge Way
Richmond, B.C. V6X 2W8
Canada

Phone: 1.604.285.2602
Fax: 1.604.285.2606
Email: sales@glglifetech.com
Web: www.glglifetech.com

Manufactured By: Qingdao Runde Biotechnology Co.,Ltd.
Lingshanwei Town, Jiaonan County,
Qingdao, Shandong, 266427 China

Phone: +86.532.83181169
Fax: +86.532.83181836

Qingdao Runde Biotechnology Co.,Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Manufacturer's FDA Registration Number:19905279852

Date of Analysis: March 15th ,2018

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Organoleptic AS IS
Particle Size	100% pass through 80 mesh	Conform	Ro Tap
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.32%	JECFA 2017
Total Steviol Glycosides (wt/wt)	≥95.0% (on dry basis)	98.78%	JECFA 2017
Loss on Drying	≤ 6.0%	2.53%	JECFA Vol.4
pH	4.5-7.0	5.5	JECFA Vol.4
Residue on Ignition	≤ 1.0%	0.09 %	JECFA Vol.4
Lead (as Pb)	≤1.0 ppm	0.02 ppm	JECFA Vol.4
Arsenic (as As)	≤1.0 ppm	0.04ppm	JECFA Vol.4
Cadmium (as Cd)	≤1.0 ppm	0.01ppm	JECFA Vol.4
Mercury (as Hg)	≤1.0 ppm	0.03ppm	JECFA Vol.4
Residual solvents -Ethanol	≤ 5000 ppm	1587.3 ppm	JECFA Vol.4
-Methanol	≤ 200 ppm	83.5 ppm	JECFA Vol.4
Total PlateCount	< 1000 CFU/g	< 10 cfu/g	FDA-BAM chapter 3
Yeast& Mold	<100 CFU/g	< 10 cfu/g	FDA-BAM chapter 18
E.coli	< 3 MPN/g	< 3 MPN/g	FDA-BAM chapter 4
Staphylococcus Aureus	< 10 CFU/g	< 10 CFU/g	FDA-BAM chapter 12
Salmonella(/25g)	Negative	Negative	FDA-BAM chapter 5

Conclusion	QUALIFIED

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: [redacted] **Date:** 15/03/2018

Checked by: [redacted] **Date:** 15/03/2018.

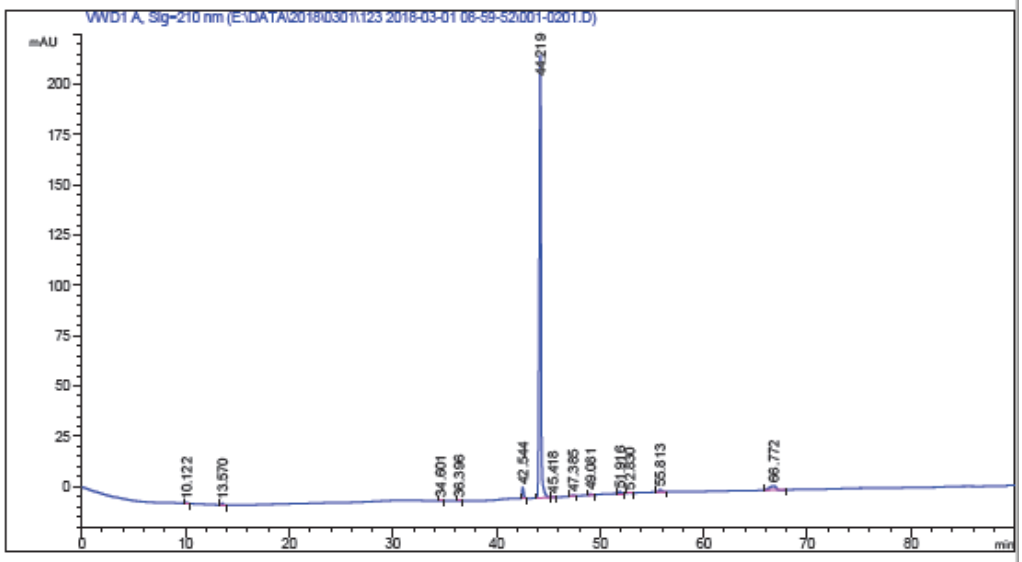
Approved by: [redacted] (Quality Manager) **Date:** 15/03/2018

Appendix 3 Analytical Chromatograms for Multiple Production Batches of Festeviol™ RM 95

Appendix 3.1 Festeviol™ RM 95 Lot [redacted]

Data File: E:\DATA\2018\0301\123 2018-03-01 08-59-52\001-0201.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-[redacted]

Acq. Operator : Sun Hongkai Seq. Line : 2
Instrument : Instrument 1 Location : 1
Injection Date : 2018-3-01 10:32:51 Inj : 1
Inj Vulme: 10.0 µl
Acq. Method : E:\DATA\2018\0301\123 2018-03-01 08-59-52\JECFA 2017.M
Last Changed : 2018-3-01 08:36:50 : Sun Hongkai
Analysis Method : D:\CHEM32\1\METHODS\JECFA 2017.M
Last Changed : 2018-3-01 15:02:40 : Sun Hongkai
(modified after loading)



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
1	10.122	BB	0.1879	4.23177	3.52924e-1	0.1454	
2	13.570	BB	0.1871	9.12156	7.90200e-1	0.3133	
3	34.601	BB	0.1693	2.04071	1.73449e-1	0.0701	
4	36.396	BB	0.1635	1.38380	1.29998e-1	0.0475	
5	42.544	BB	0.1768	62.06179	5.49893	2.1317	Rebaudioside D
6	44.219	BB	0.1895	2687.81763	219.49648	92.3222	Rebaudioside M
7	45.418	BB	0.1953	6.49254	4.90271e-1	0.2230	
8	47.385	BB	0.2160	7.17210	4.93929e-1	0.2464	

Instrument 1 2018-3-01 15:04:17 Sun Hongkai Page 1/2

Data File: E:\DATA\2018\0301\123 2018-03-01 08-59-52\001-0201.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-[redacted]

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
9	49.081	BB	0.1828	4.92217	4.04392e-1	0.1691	
10	51.916	BB	0.2204	11.80791	8.06152e-1	0.4056	Rebaudioside A
11	52.830	BB	0.2995	4.99170	2.35667e-1	0.1715	Stevioside
12	55.813	BB	0.3296	26.30150	1.22256	0.9034	Rebaudioside C
13	66.772	BB	0.5917	82.99886	1.97704	2.8509	Rebaudioside B
Totals:				2911.34404	232.07199		

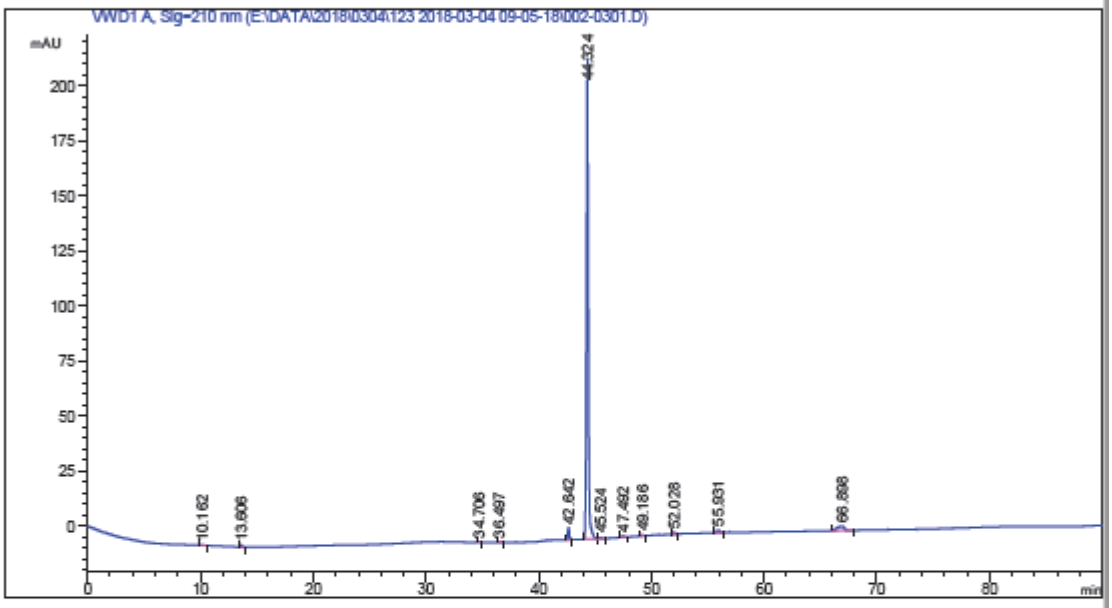
*** End of Report ***

Instrument 1 2018-3-01 15:04:17 Sun Hongkai Page 2/2

Appendix 3.2 Festeviol™ RM 95 Lot

Data File: E:\DATA\2018\0304\123 2018-03-04 09-05-18\002-0301.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-

Acq. Operator	: Sun Hongkai	Seq. Line :	3
Instrument	: Instrument 1	Location :	2
Injection Date	: 2018-3-04 10:38:17	Inj :	1
		Inj Vulme: 10.0 µl	
Acq. Method	: E:\DATA\2018\0304\123 2018-03-04 09-05-18\JECFA 2017.M		
Last Changed	: 2018-3-04 08:23:50 : Sun Hongkai		
Analysis Method	: D:\CHEM32\1\METHODS\JECFA 2017.M		
Last Changed	: 2018-3-04 13:32:22 : Sun Hongkai		
	(modified after loading)		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
1	10.162	BB	0.1935	4.22237	3.49183e-1	0.1459	
2	13.606	BB	0.1839	9.50550	7.99441e-1	0.3284	
3	34.706	BB	0.1761	2.09497	1.76743e-1	0.0724	
4	36.497	BB	0.1891	1.53419	1.32333e-1	0.0530	
5	42.642	BB	0.1752	61.46252	5.45220	2.1236	Rebaudioside D
6	44.324	BB	0.1868	2677.27979	218.17667	92.5014	Rebaudioside M
7	45.524	BB	0.1874	6.31079	4.92262e-1	0.2180	
8	47.492	BB	0.2075	6.99024	4.89174e-1	0.2415	

Instrument 1 2018-3-04 13:34:46 Sun Hongkai Page 1/2

Data File: E:\DATA\2018\0304\123 2018-03-04 09-05-18\002-0301.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-20180304

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
9	49.186	BB	0.1845	4.92875	4.08387e-1	0.1703	
10	52.028	BB	0.2118	10.56998	7.73661e-1	0.3652	Rebaudioside A
11	55.931	BB	0.3361	26.74925	1.22576	0.9242	Rebaudioside C
12	66.898	BB	0.5928	82.66410	2.00035	2.8561	Rebaudioside B
Totals:				2894.31246	230.47616		

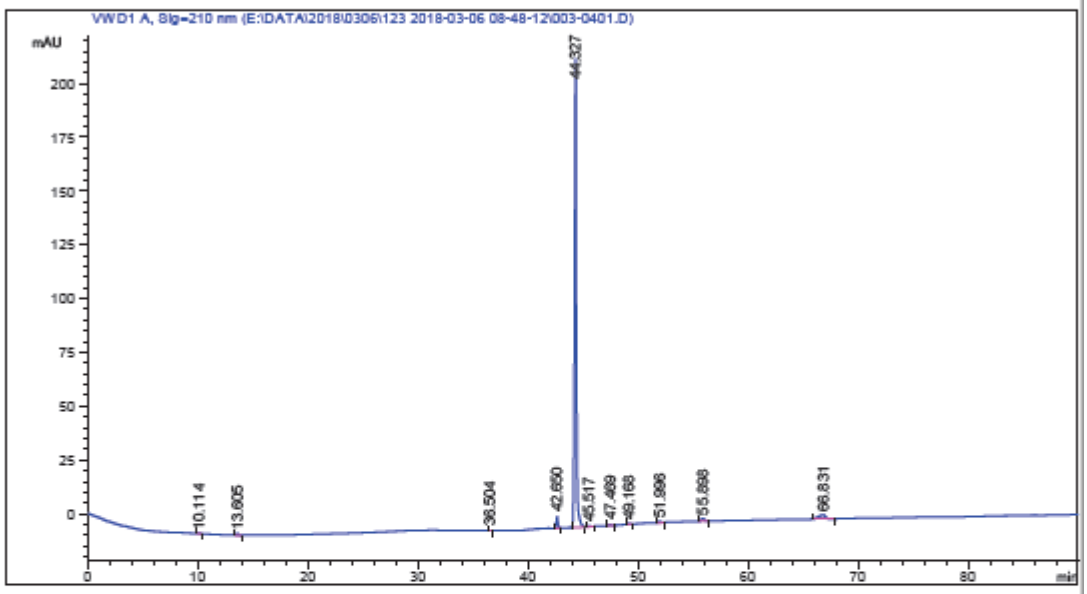
*** End of Report ***

Instrument 1 2018-3-04 13:34:46 Sun Hongkai Page 2/2

Appendix 3.3 Festeviol™ RM 95 Lot [redacted]

Date File: E:\DATA\2018\0306\123 2018-03-06 08-48-12\003-0401.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-[redacted]

Acq. Operator	: Sun Hongkai	Seq. Line :	4
Instrument	: Instrument 1	Location :	3
Injection Date	: 2018-3-06 10:25:20	Inj :	1
		Inj Vulme:	10.0 µl
Acq. Method	: E:\DATA\2018\0306\123 2018-03-06 08-48-12\JECFA 2017.M		
Last Changed	: 2018-3-06 08:40:43 : Sun Hongkai		
Analysis Method	: D:\CHEM32\1\METHODS\JECFA 2017.M		
Last Changed	: 2018-3-06 13:23:29 : Sun Hongkai		
	(modified after loading)		



Area Percent Report

Sorted By	:	Signal
Multiplier:	:	1.0000
Dilution:	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
1	10.114	BB	0.1905	4.22512	3.45997e-1	0.1470	
2	13.605	BB	0.1860	9.05615	7.91077e-1	0.3152	
3	36.504	BB	0.1400	1.35760	1.35664e-1	0.0472	
4	42.650	BB	0.1735	61.34915	5.45068	2.1351	Rebaudioside D
5	44.327	BB	0.1891	2662.21631	217.94154	92.6531	Rebaudioside M
6	45.517	BB	0.1942	6.16359	4.87013e-1	0.2145	
7	47.469	BB	0.2080	7.07010	4.93217e-1	0.2461	
8	49.168	BB	0.1854	4.81760	3.96596e-1	0.1677	

Instrument 1 2018-3-06 13:24:34 Sun Hongkai Page 1/2

Date File: E:\DATA\2018\0306\123 2018-03-06 08-48-12\003-0401.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-20180306

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
9	51.996	BB	0.2024	9.46529	6.95567e-1	0.3294	Rebaudioside A
10	55.898	BB	0.3161	25.87030	1.21146	0.9004	Rebaudioside C
11	66.831	BB	0.5781	81.72430	1.99006	2.8443	Rebaudioside B
Totals:				2873.31552	229.93887		

*** End of Report ***

Instrument 1 2018-3-06 13:24:34 Sun Hongkai Page 2/2

Appendix 3.4 Festeviol™ RM 95 Lot

Data File: E:\DATA\2018\0308\123 2018-03-08 08-50-27\004-0501.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-[redacted]

```
==========================================================================
Acq. Operator   : Sun Hongkai                    Seq. Line :   5
Instrument      : Instrument 1                    Location :   4
Injection Date  : 2018-3-08 10:23:30              Inj      :   1
                                                  Inj Vulme: 10.0 µl
Acq. Method     : E:\DATA\2018\0308\123 2018-03-08 08-50-27\JECFA 2017.M
Last Changed    : 2018-3-08 08:31:05 : Sun Hongkai
Analysis Method : D:\CHEM32\1\METHODS\JECFA 2017.M
Last Changed    : 2018-3-08 13:44:01 :  Sun Hongkai
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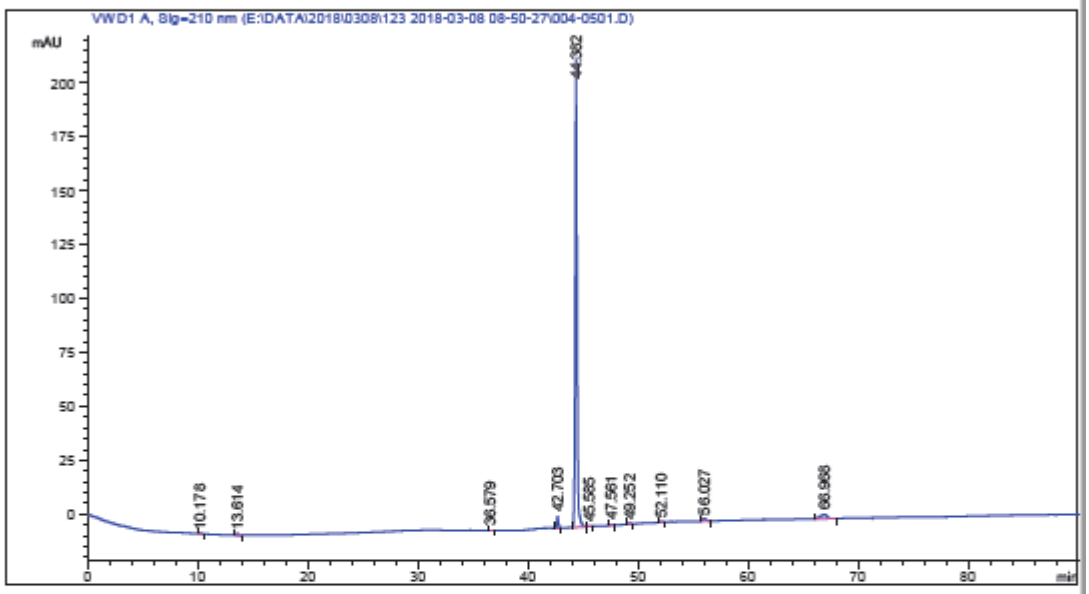



```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By                 :      Signal
Multiplier:               :      1.0000
Dilution:                 :      1.0000
Use Multiplier & Dilution Factor with ISTDs


 Signal 1: VWD1 A, Sig=210 nm
```

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
1	10.178	BB	0.1878	4.11020	3.43064e-1	0.1427	
2	13.614	BB	0.1866	9.40420	8.17640e-1	0.3264	
3	36.579	BB	0.1766	1.49616	1.34223e-1	0.0519	
4	42.703	BB	0.1768	61.47449	5.44906	2.1339	Rebaudioside D
5	44.382	BB	0.1899	2669.43750	217.26897	92.6619	Rebaudioside M
6	45.585	BB	0.1941	6.49869	4.94388e-1	0.2256	
7	47.561	BB	0.2030	6.86277	4.84705e-1	0.2382	
8	49.252	BB	0.1805	4.83774	3.95808e-1	0.1679	

```
Instrument 1 2018-3-08 13:46:07 Sun Hongkai                    Page 1/2
```

Data File: E:\DATA\2018\0308\123 2018-03-08 08-50-27\004-0501.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-20180308

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
9	52.110	BB	0.2136	9.38034	6.78865e-1	0.3256	Rebaudioside A
10	56.027	BB	0.3101	25.95315	1.21647	0.9009	Rebaudioside C
11	66.968	BB	0.5628	81.38086	1.99693	2.8249	Rebaudioside B
Totals:				2880.83610	229.28012		

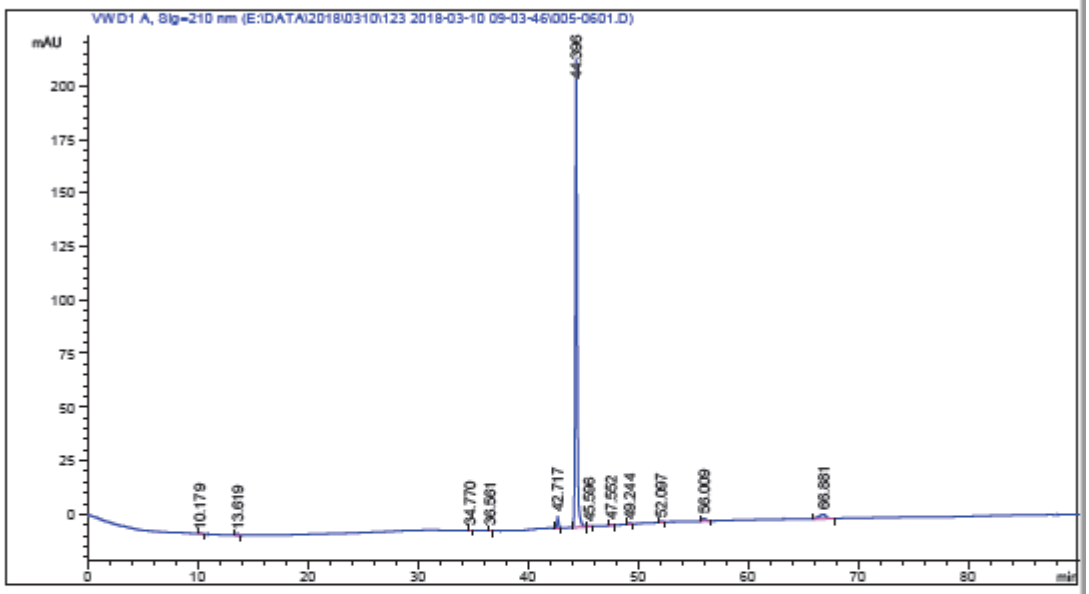
*** End of Report ***

Instrument 1 2018-3-08 13:46:07 Sun Hongkai Page 2/2

Appendix 3.5 Festeviol™ RM 95 Lot

Data File: E:\DATA\2018\0310\123 2018-03-10 09-03-46\005-0601.D
Sample Name: GLG Festeviol RM95/ GLG-RM95

Acq. Operator	: Sun Hongkai	Seq. Line :	6
Instrument	: Instrument 1	Location :	5
Injection Date	: 2018-3-10 10:36:45	Inj :	1
		Inj Vulme:	10.0 µl
Acq. Method	: E:\DATA\2018\0310\123 2018-03-10 09-03-46\JECFA 2017.M		
Last Changed	: 2018-3-10 08:54:18 : Sun Hongkai		
Analysis Method	: D:\CHEM32\1\METHODS\JECFA 2017.M		
Last Changed	: 2018-3-10 13:40:22 : Sun Hongkai		
	(modified after loading)		



Area Percent Report

Sorted By	:	Signal
Multiplier:	:	1.0000
Dilution:	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
1	10.179	BB	0.2004	4.24318	3.41132e-1	0.1468	
2	13.619	BB	0.1827	9.70944	8.23527e-1	0.3358	
3	34.770	BB	0.1538	1.83561	1.75839e-1	0.0635	
4	36.561	BB	0.1540	1.34249	1.31586e-1	0.0464	
5	42.717	BB	0.1733	61.58346	5.48002	2.1299	Rebaudioside D
6	44.396	BB	0.1894	2676.32788	218.62177	92.5643	Rebaudioside M
7	45.596	BB	0.1991	6.38969	4.88936e-1	0.2210	
8	47.552	BB	0.2097	6.98433	4.90949e-1	0.2416	

Instrument 1 2018-3-10 13:42:06 Sun Hongkai Page 1/2

Data File: E:\DATA\2018\0310\123 2018-03-10 09-03-46\005-0601.D
Sample Name: GLG Festeviol RM95/ GLG-RM95-20180310

Peak #	Ret Time [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %	Name
9	49.244	BB	0.1960	4.98057	3.96561e-1	0.1723	
10	52.097	BB	0.2127	9.21009	6.70326e-1	0.3185	Rebaudioside A
11	56.009	BB	0.3148	26.02364	1.22502	0.9001	Rebaudioside C
12	66.881	BB	0.5741	82.68728	2.04285	2.8598	Rebaudioside B
Totals:				2891.31767	230.88852		

*** End of Report ***

Instrument 1 2018-3-10 13:42:06 Sun Hongkai Page 2/2

Appendix 4 Solubility Testing Report

GLG LIFE TECH CORPORATION	Issue Date:10/09/2018
GLG Solubility Report of Steviol Glycosides/ GLG Festiviol™ RM95	File No: GLG-QA-SSD-RM95-S

GLG Solubility Report of Steviol Glycosides/ GLG Festiviol™ RM95

Prepared by: Zhang Lei (QA/QC Manager, GLG Life Tech Corporation)

Date: 10/09/2018

Approved by: Kevin Li (VP of Innovation and QA, GLG Life Tech Corporation)

Date: 10/09/2018

1 / 3

GLG LIFE TECH CORPORATION	Issue Date:10/09/2018
GLG Solubility Report of Steviol Glycosides/ GLG Festiviol™ RM95	File No: GLG-QA-SSD-RM95-S

Objective

To determine solubility of Steviol Glycosides / GLG Festiviol™ RM95 produced by GLG.

Samples

On sample representing commercial lot of Steviol Glycosides / GLG FestiviolTM RM95 labeled as "GLG-RM95- GLG-RM95- GLG-RM95- GLG-RM95- GLG-RM95-

Solubility Criteria

USP & IP Solubility Criteria

Description	Parts of solvent required for one part of solute
Very soluble	< 1
Freely soluble	1 - 10
Soluble	10 - 30
Sparingly soluble	30 - 100
Slightly soluble	100 - 1000
Very slightly soluble	1000 - 10,000
Insoluble	> 10,000

Experimental condition

Temperature: 20°C
Solvent: Water: Ethanol 50:50

Experimental procedure

1, Weigh 30g of the above 5 samples separately;

2 / 3

2, Separately add a small amount of 5 samples to 5 beakers which containing 100ml of solvent (water: ethanol 50:50);
3, Separately shaking the 5 beakers for 1 minute, if the solution is clear, continue to add a small amount of samples;
4, Stop adding sample until the solution is no longer clear;
5, Weigh the remaining of each sample and calculate the solubility.
Samples solubility are as follows:

Samples	Solubility, g/100ml（Water: Ethanol 50:50）
GLG-RM95-	15.5
GLG-RM95-	16.9
GLG-RM95-	13.1
GLG-RM95-	18.3
GLG-RM95-	15.4
Average	15.8

Experimental Conclusion

GLG Festiviol™ RM95 can freely soluble in the solvent that water: ethanol 50:50.

Appendix 5 Pesticide Testing Report

SGS

Test Report QDF18-030467-02 Date: 30 Sep 2016

Client Name: Qingdao Runde Biotechnology Co.,Ltd
Client Address: Lingshanwei, Jiaonan, Qingdao, Shandong 266427 China

Sample Name:	Steviol glycosides / GLG Festeviol™RebM 95
Manufacturer:	Qingdao Runde Biotechnology Co.,Ltd
Sample Batch No.:	GLG-RM95-[redacted]
Production Date:	/

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS Reference No. :	TAOFD1804603501
Date of Sample Received :	26 Sep 2016
Testing Period :	26 Sep 2016 - 30 Sep 2016
Test Requested :	Selected test(s) as requested by client.
Test Method :	Please refer to next page(s).
Test Result(s) :	Please refer to next page(s).

Chinese shall prevail in this report.

[redacted]

SGS Authorized signature

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SGS

Test Report

QDF18-030467-02 Date: 30 Sep 2018

Sample Description :

Specimen No.	SGS Sample ID	Description
1	QDF18-030467.001	sample in bag

Chemical test

Test Result(s) :

Test Item(s)	Unit	Test Method(s)	Test Result(s) 001	LOQ
Lead(Pb)	mg/kg	GB 5009.12-2017 I	ND	0.04
Total Arsenic (As)	mg/kg	GB 5009.11-2014 I	0.013	0.01
Cadmium (Cd)	mg/kg	GB 5009.15-2014	0.010	0.003
Total Mercury (Hg)	mg/kg	GB 5009.17-2014 I	ND	0.01

Pesticide residues

Test Method(s) : With reference to §64 LFGB L 00.00-34 (2010). Analysis was conducted with GC-MS,LC-MS-MS

Test Result(s) :

Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
1	2-phenyl-phenol 邻苯基苯酚	mg/kg	90-43-7	ND	0.01
2	Acephate 乙酰甲胺磷	mg/kg	30560-19-1	ND	0.01
3	Acetamiprid 啶虫脒	mg/kg	135410-20-7	ND	0.01
4	Acetochlor 乙草胺	mg/kg	34256-82-1	ND	0.01
5	a-HCH a-六六六	mg/kg	319-84-6	ND	0.01
6	Aldicarb 涕灭威	mg/kg	116-06-3	ND	0.01
7	Aldicarb-sulfoxid 涕灭威亚砜	mg/kg	1646-87-3	ND	0.01
8	Aldoxycarb 铁灭威	mg/kg	1646-88-4	ND	0.01
9	Atrazine 莠去津	mg/kg	1912-24-9	ND	0.01
10	Azinphos-methyl 保棉磷	mg/kg	86-50-0	ND	0.01
11	Azoxystrobin 嘧菌酯	mg/kg	131860-33-8	ND	0.01
12	Benalaxyl & Benalaxyl-M 苯霜灵和精苯霜灵	mg/kg	71626-11-4 & 98243-83-6	ND	0.01
13	Bendiocarb 恶虫威	mg/kg	22781-23-3	ND	0.01
14	Benfluralin 乙丁氟灵	mg/kg	1861-40-1	ND	0.01
15	Benfuracarb 丙硫克百威	mg/kg	82560-54-1	ND	0.01
16	Benoxacor 解草嗪	mg/kg	98730-04-2	ND	0.01

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Test Report GDF18-030457-02 Date: 30 Sep 2018

Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
17	Bensulfuron-methyl 苄嘧磺隆	mg/kg	83055-99-6	ND	0.01
18	Bifenthrin 联苯菊酯	mg/kg	82657-04-3	ND	0.01
19	Boscalid 啶酰菌胺	mg/kg	188425-85-6	ND	0.01
20	Bromopropylate 溴螨酯	mg/kg	18181-80-1	ND	0.01
21	Bupirimate 乙嘧酚磺酸酯	mg/kg	41483-43-6	ND	0.01
22	Buprofezin 噻嗪酮	mg/kg	69327-76-0	ND	0.01
23	Butachlor 丁草胺	mg/kg	23184-66-9	ND	0.01
24	Butocarboxim 丁酮威	mg/kg	034681-10-2	ND	0.01
25	Cadusafos 硫线磷	mg/kg	95465-99-9	ND	0.01
26	Captan克菌丹	mg/kg	133-06-2	ND	0.10
27	Carbaryl 甲萘威	mg/kg	63-25-2	ND	0.01
28	Carbendazim 多菌灵	mg/kg	10605-21-7	ND	0.01
29	Carbofuran 虫螨威（克百威）	mg/kg	1563-66-2	ND	0.01
30	Carbofuran-3-hydroxy 3-羟基虫螨威	mg/kg	16655-82-6	ND	0.01
31	Carbosulfan 丁硫克百威	mg/kg	55285-14-8	ND	0.01
32	Chlorbenzuron 灭幼脲	mg/kg	57160-47-1	ND	0.01
33	Chlordane 克氯丹	mg/kg	57-74-9	ND	0.01
34	Chlorfenapyr 虫螨腈	mg/kg	122453-73-0	ND	0.01
35	Chlorfenvinphos 毒虫畏	mg/kg	470-90-6	ND	0.01
36	Chlorpropham 氯苯胺灵	mg/kg	101-21-3	ND	0.01
37	Chlorpyrifos Methyl 甲基毒死蜱	mg/kg	5598-13-0	ND	0.01
38	Chlorpyrifos 毒死蜱	mg/kg	2921-88-2	ND	0.01
39	Clethodim 烯草酮	mg/kg	99129-21-2	ND	0.01
40	Clothianidin 噻虫胺/可尼丁	mg/kg	210880-92-5	ND	0.01
41	Cyanazine 氰草津	mg/kg	21725-46-2	ND	0.01
42	Cyflufenamid 环氟菌胺	mg/kg	180409-60-3	ND	0.01
43	Cyfluthrin 氟氯氰菊酯	mg/kg	68359-37-5	ND	0.01
44	Cymoxanil 霜脲氰	mg/kg	57966-95-7	ND	0.01
45	Cypermethrin 氯氰菊酯	mg/kg	52315-07-8	ND	0.01
46	Cyprodinil 嘧菌环胺	mg/kg	121552-61-2	ND	0.01
47	Cyromazine 灭蝇胺	mg/kg	66215-27-8	ND	0.01
48	Deltamethrin & Tralomethrin 溴氰菊酯和四溴菊酯	mg/kg	52918-63-5 & 66841-25-6	ND	0.01
49	Diazinon 二嗪磷	mg/kg	333-41-5	ND	0.01
50	Dichlorvos 敌敌畏	mg/kg	62-73-7	ND	0.01
51	Dicloran 氯硝胺	mg/kg	99-30-9	ND	0.01
52	Dicofol 三氯杀螨醇	mg/kg	115-32-2	ND	0.01

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Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
53	Diethofencarb 乙霉威	mg/kg	87130-20-9	ND	0.01
54	Difenoconazole 苯醚甲环唑	mg/kg	119446-68-3	ND	0.01
55	Dimethoate 乐果	mg/kg	60-51-5	ND	0.01
56	Dimethomorph 烯酰吗啉	mg/kg	110488-70-5	ND	0.01
57	Diniconazole 烯唑醇	mg/kg	83657-24-3	ND	0.01
58	Edifenphos 敌瘟磷	mg/kg	17109-49-8	ND	0.01
59	Emamectin benzoate 甲胺基阿维菌素苯甲酸盐	mg/kg	155569-91-8	ND	0.01
60	Endosulfan-sulfate 硫丹硫酸酯	mg/kg	1031-07-8	ND	0.01
61	Ethiofencarb 乙硫甲威	mg/kg	29973-13-5	ND	0.01
62	Ethion 乙硫磷	mg/kg	563-12-2	ND	0.01
63	Ethoprophos 灭线磷	mg/kg	13194-48-4	ND	0.01
64	Etofenprox 醚菊酯	mg/kg	80844-07-1	ND	0.01
65	Etrimfos 乙嘧硫磷	mg/kg	38260-54-7	ND	0.01
66	Famoxadone 恶唑菌酮	mg/kg	131807-57-3	ND	0.05
67	Fenarimol 氯苯嘧啶醇	mg/kg	60168-88-9	ND	0.01
68	Fenhexamid 环酰菌胺	mg/kg	126833-17-8	ND	0.01
69	Fenitrothion 杀螟硫磷	mg/kg	122-14-5	ND	0.01
70	Fenobucarb 仲丁威	mg/kg	3766-81-2	ND	0.01
71	Fenoxycarb 苯氧威	mg/kg	79127-80-3	ND	0.01
72	Fenpropathrin 甲氰菊酯	mg/kg	64257-84-7	ND	0.01
73	Fenpropimorph 丁苯吗啉	mg/kg	67564-91-4	ND	0.01
74	Fenpyroximate 唑螨酯	mg/kg	111812-58-9	ND	0.01
75	Fenthion 倍硫磷	mg/kg	55-38-9	ND	0.01
76	Fenvalerate & Esfenvalerate 氰戊菊酯和高效氰戊菊酯	mg/kg	51630-58-1&66230-04-4	ND	0.01
77	Fipronil 氟虫腈	mg/kg	120068-37-3	ND	0.01
78	Fluazifop-butyl & Fluazifop-p-butyl 吡氟禾草灵和精吡氟禾草灵	mg/kg	69806-50-4 & 79241-46-6	ND	0.01
79	Flucythrinate 氟氰戊菊酯	mg/kg	70124-77-5	ND	0.01
80	Flufenoxuron 氟虫脲	mg/kg	101463-69-8	ND	0.01
81	Flusilazole 氟硅唑	mg/kg	85509-19-9	ND	0.01
82	Furathiocarb 呋线威	mg/kg	65907-30-4	ND	0.01
83	Haloxyfop-methyl 氟吡甲禾灵	mg/kg	69806-40-2	ND	0.01
84	Heptenophos 庚烯磷	mg/kg	23560-59-0	ND	0.01
85	Hexythiazox 噻螨酮	mg/kg	78587-05-0	ND	0.01
86	Imazalil 抑霉唑/咪鲜灵	mg/kg	35554-44-0	ND	0.01

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Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
87	Imidaclopnd 吡虫啉	mg/kg	138261-41-3	ND	0.01
88	Indoxacarb 茚虫威（安打）	mg/kg	173584-44-6	ND	0.01
89	Iprodione 异菌脲	mg/kg	36734-19-7	ND	0.01
90	Iprovalicarb 丙森锌	mg/kg	140923-17-7	ND	0.01
91	Isocarbophos 水胺硫磷	mg/kg	24353-61-5	ND	0.01
92	Isofenphos 异柳磷	mg/kg	25311-71-1	ND	0.01
93	Isofenphos-methyl 甲基异柳磷	mg/kg	99675-03-3	ND	0.01
94	Isoprocarb 异丙威	mg/kg	2631-40-5	ND	0.01
96	Isoprothiolane 稻瘟灵	mg/kg	50512-35-1	ND	0.01
96	Isoproturon 异丙隆	mg/kg	34123-59-6	ND	0.01
97	Kresoxim-methyl 苯氧菌胺/克肟菌	mg/kg	143390-89-0	ND	0.01
98	Linuron 利谷隆	mg/kg	330-55-2	ND	0.01
99	Malathion 马拉硫磷	mg/kg	121-75-5	ND	0.01
100	Metalaxyl & Metalaxyl-M 甲霜灵和精甲霜灵	mg/kg	57837-19-1 & 70630-17-0	ND	0.01
101	Metamitron 苯嗪草	mg/kg	41394-05-2	ND	0.01
102	Methamidophos 甲胺磷	mg/kg	10265-92-6	ND	0.01
103	Methidathion 杀扑磷	mg/kg	950-37-8	ND	0.01
104	Methiocarb 灭虫威	mg/kg	2032-65-7	ND	0.01
105	Methomyl 灭多威	mg/kg	16752-77-5	ND	0.01
106	Methoxyfenozide 甲氧虫酰肼	mg/kg	161050-58-4	ND	0.01
107	Metolachlor & S-Metolachlor 异丙甲草胺和精-异丙甲草胺	mg/kg	51218-45-2 & 87392-12-9	ND	0.01
108	Mevinphos 速灭磷	mg/kg	7786-34-7	ND	0.01
109	Monocrotophos 久效磷	mg/kg	6923-22-4	ND	0.01
110	Myclobutanil 灭克萎/腈菌唑	mg/kg	88671-89-0	ND	0.01
111	Napropamide 敌草胺	mg/kg	15299-99-7	ND	0.01
112	Nicosulfuron 烟嘧磺隆	mg/kg	111991-09-4	ND	0.01
113	Nitrothal-Isopropyl 稻瘟酯	mg/kg	10552-74-6	ND	0.01
114	o,p'-DDD o,p'-滴滴滴	mg/kg	53-19-0	ND	0.01
115	o,p'-DDE o,p'-滴滴伊	mg/kg	3424-82-6	ND	0.01
116	o,p'-DDT o,p'-滴滴涕	mg/kg	789-02-6	ND	0.01
117	Omethoate 氧化乐果	mg/kg	1113-02-6	ND	0.01
118	Oxadiazon 噁草酮	mg/kg	19666-30-9	ND	0.01
119	Oxadixyl 恶霜灵	mg/kg	77732-09-3	ND	0.01
120	Oxy-Chlordane 氧氯丹	mg/kg	27304-13-8	ND	0.01
121	Oxydemeton-methyl 砜吸磷	mg/kg	301-12-2	ND	0.01

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Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
122	p,p'-DDD p,p'-滴滴滴	mg/kg	72-54-8	ND	0.01
123	p,p'-DDE p,p'-滴滴伊	mg/kg	72-55-9	ND	0.01
124	p,p'-DDT p,p'-滴滴涕	mg/kg	50-29-3	ND	0.01
125	Paclobutrazol 多效唑	mg/kg	76738-62-0	ND	0.01
126	Parathion Methyl 甲基对硫磷	mg/kg	298-00-0	ND	0.01
127	Parathion 对硫磷	mg/kg	56-38-2	ND	0.01
128	Penconazole 戊菌唑	mg/kg	66246-88-6	ND	0.01
129	Pendimethalin 二甲戊乐灵	mg/kg	40487-42-1	ND	0.01
130	Permethrin 氯菊酯	mg/kg	52645-53-1	ND	0.01
131	Phenthoate 稻丰散	mg/kg	2697-03-7	ND	0.01
132	Phorate sulfone 甲拌磷砜	mg/kg	2588-04-7	ND	0.05
133	Phorate sulfoxide 甲拌磷亚砜	mg/kg	2588-03-6	ND	0.05
134	Phorate 甲拌磷	mg/kg	298-02-2	ND	0.01
135	Phosalone 伏杀硫磷	mg/kg	2310-17-0	ND	0.01
136	Phosmet 亚胺硫磷	mg/kg	732-11-6	ND	0.01
137	Phosphamidon 磷胺	mg/kg	13171-21-6	ND	0.05
138	Phoxim 辛硫磷	mg/kg	14816-18-3	ND	0.01
139	Pirimicarb 抗蚜威	mg/kg	23103-98-2	ND	0.01
140	Pirimiphos-Ethyl 嘧啶磷	mg/kg	23505-41-1	ND	0.01
141	Pirimiphos-Methyl 甲基嘧啶磷	mg/kg	29232-93-7	ND	0.01
142	Prochloraz 咪鲜胺	mg/kg	67747-09-5	ND	0.01
143	Procymidone 腐霉利	mg/kg	32809-16-8	ND	0.01
144	Profenophos 四氯磷	mg/kg	41198-08-7	ND	0.01
145	Promecarb 猛杀威	mg/kg	2631-37-0	ND	0.01
146	Prometryn 扑草净	mg/kg	7287-19-6	ND	0.01
147	Propamocarb 霜霉威	mg/kg	24579-73-5	ND	0.01
148	Propargite 炔螨特	mg/kg	2312-35-8	ND	0.01
149	Propham 苯胺灵	mg/kg	122-42-9	ND	0.01
150	Propiconazole 丙环唑	mg/kg	60207-90-1	ND	0.01
151	Propoxur 残杀威	mg/kg	114-26-1	ND	0.01
152	Propyzamide 炔苯酰草胺	mg/kg	23950-58-5	ND	0.01
153	Pymetrozine 吡蚜酮/吡嗪酮	mg/kg	123312-89-0	ND	0.01
154	Pyrazophos 吡菌磷	mg/kg	13457-18-6	ND	0.01
155	Pyridaben 哒螨灵/哒螨酮	mg/kg	96489-71-3	ND	0.01
156	Pyridaphenthion 哒嗪硫磷	mg/kg	119-12-0	ND	0.01
157	Pyrimethanil 嘧霉胺	mg/kg	53112-28-0	ND	0.01
158	Quinalphos 喹硫磷	mg/kg	13593-03-8	ND	0.01
159	Quintozene 五氯硝基苯	mg/kg	82-68-8	ND	0.01

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Code	Test Item(s)	Unit	CAS_NO	Test Result(s) 001	LOQ
160	Quizalofop-ethyl 喹禾灵 Quizalofop-p-ethyl 喹禾灵/禾草克和精喹禾灵	mg/kg	76578-14-8 & 100646-51-3	ND	0.01
161	Rimsulfuron 砜嘧磺隆	mg/kg	122931-48-0	ND	0.01
162	S-421 八氯二苯醚	mg/kg	127-90-2	ND	0.01
163	Simazine 西玛津	mg/kg	122-34-9	ND	0.01
164	Spinosad 艾克敌(多杀菌素)	mg/kg	168316-95-8	ND	0.01
165	Spiroxamine 螺环菌胺	mg/kg	118134-30-8	ND	0.01
166	Tau-Fluvalinate 氟胺氰菊酯	mg/kg	102851-06-9	ND	0.05
167	Tebuconazole 戊唑醇	mg/kg	107534-96-3	ND	0.01
168	Tebufenozide 虫酰肼	mg/kg	112410-23-8	ND	0.01
169	Tetrachlorvinphos 杀虫畏	mg/kg	22248-79-9	ND	0.01
170	Tetradifon 四氯杀螨砜	mg/kg	116-29-0	ND	0.01
171	Thiabendazole 噻菌灵	mg/kg	148-79-8	ND	0.01
172	Thiacloprid 噻虫啉	mg/kg	111988-49-9	ND	0.01
173	Thiamethoxam 噻虫嗪	mg/kg	153719-23-4	ND	0.01
174	Thifensulfuron-methyl 噻吩磺隆	mg/kg	79277-27-3	ND	0.01
175	Thiodicarb 硫双威	mg/kg	59669-26-0	ND	0.01
176	Thiofanox-sulfon 久效威砜	mg/kg	39184-59-3	ND	0.01
177	Thiofanox-sulfoxid 久效威亚砜	mg/kg	39184-27-5	ND	0.01
178	Tolclofos-methyl 甲基立枯磷	mg/kg	57018-04-9	ND	0.01
179	Triadimefon 三唑酮	mg/kg	43121-43-3	ND	0.01
180	Triadimenol 三唑醇	mg/kg	55219-65-3	ND	0.01
181	Triasulfuron 醚苯磺隆	mg/kg	82097-50-5	ND	0.01
182	Triazophos 三唑磷	mg/kg	24017-47-8	ND	0.01
183	Trichlorphon 敌百虫	mg/kg	52-68-6	ND	0.01
184	Triflumizole 氟菌唑	mg/kg	68694-11-1	ND	0.01
185	Trifluralin 氟乐灵	mg/kg	1582-09-8	ND	0.01
186	Triflusulfuron-methyl 氟胺磺隆	mg/kg	126535-15-7	ND	0.01
187	Vamidothion 完灭硫磷	mg/kg	2275-23-2	ND	0.01
188	Vinclozoline 乙烯菌核利	mg/kg	50471-44-8	ND	0.01
189	α-endosulfan α-硫丹	mg/kg	959-98-8	ND	0.01
190	β-endosulfan β-硫丹	mg/kg	33213-65-9	ND	0.01
191	β-HCH β-六六六	mg/kg	319-85-7	ND	0.01
192	γ-HCH/lindane γ-六六六/林丹	mg/kg	58-89-9	ND	0.01
193	δ-HCH δ-六六六	mg/kg	319-86-8	ND	0.01
194	λ-Cyhalothrin 高效氯氟氰菊酯	mg/kg	91465-08-6	ND	0.01

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Test Report QDF18-030467-02 Date: 30 Sep 2018

Remark:
1.ND = Not Detected
2.LOQ = Limit of Quantitation

*** End ***

Appendix 6 Residual Protein Testing Report

SGS

Test Report QDF18-030552-01 Date: 30 Sep 2018

Client Name: Qingdao Runde Biotechnology Co.,Ltd
Client Address: Lingshanwei Town, Jiaonan County, Qingdao, Shandong China

Sample Name:	Steviol glycosides / GLG Festeviol™ RebM 95
Manufacturer:	Qingdao Runde Biotechnology Co.,Ltd
Sample Batch No.:	GLG-RM95-
Production Date:	/

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

Date of Sample Received :	28 Sep 2018
Testing Period :	28 Sep 2018 - 30 Sep 2018
Test Requested :	Selected test(s) as requested by client.
Test Method :	Please refer to next page(s).
Test Result(s) :	Please refer to next page(s).

SGS Authorized signature

SGS-CSTC Standards Technical Services (Qingdao) Co., Ltd.
Page 1 of 2

SGS

Test Report

QDF18-030662-01 Date: 30 Sep 2018

Sample Description :

Specimen No.	SGS Sample ID	Description
1	QDF18-030662.001	sample in bag

Chemical test

Test Result(s) :

Test Item(s)	Unit	Test Method(s)	Test Result(s) 001	LOQ
Protein	%	GB 5009.5-2016 I	0.41	-

Remark:
1.Conversion factor of nitrogen to protein is 6.25
2.LOQ = Limit of Quantitation

*** End ***

Appendix 7 Relative Sweetness Intensity Method

GLG LIFE TECH CORPORATION	Issue Date:10/09/2018
Sweetness Intensity Data of Steviol Glycosides RM95	File No: GLG-QA-SSD-RM95-B

Objective

To determine sweetness intensity of Festeviol™ RM95 produced by GLG Life Tech Corporation

Samples

Samples representing commercial lot of Festeviol™ RM95 labeled as "GLG-RM95-[redacted]"

Solvents and Reagents

- Sucrose
- Festeviol™ RM 95
- Purified water
- Unsalted crackers

Apparatus

1. Analytical balance, XS205, (Mettler Toledo, USA);
2. Volumetric (class A) and Laboratory glassware, Plastic cups.

Assay and Procedures

The sweetness intensity tests are following with "ISO 8587:2006 Sensory Analysis-Methodology-Ranking" testing method.
28 panelists have been previously qualified for taste acuity and trained for the sweetness intensity test. The panelists were presented with 2 samples (5.0% of sucrose water solution and RM95 water solutions).

Test Results

Test results, see Table 1.

Table 1, Sweetness Potency of Steviol Glycoside RM95

Sample	Steviol glycosides concentration % (Sweetness equivalent to 5.0% of sucrose at 20 °C)	Sweetness Intensity
Festeviol™ RM95	0.025%	Sweetness: The sweetness equivalence of Festeviol™ RM95 solution compared to the solution was determined to be 200 times sweeter than sucrose.

Appendix 8 Estimated Daily Intake Levels of Steviol Glycosides Preparations

There have been continuing studies to estimate the intake of steviol glycosides. Most recently, Dewinter et al. (2016) investigated the dietary intake of non-nutritive sweeteners, including steviol glycosides, in children with type 1 diabetes. Using a phased tier approach, the tier 2 (maximum concentration) and tier 3 (maximum used concentrations) exposures were assessed based on survey data obtained from patients at the Pediatrics Department of the University Hospitals Leuven (Belgium). In both tier 2 and tier 3 exposure assessments, high consumers (P95) aged 4-6 years old were estimated to have a steviol glycosides intake higher than the ADI, calculated at 119% of ADI. The authors noted that the exposure assessment is a worst-case scenario since "it is assumed that all processed foods in which the food additive is authorized contain the food additive at the [maximum permitted levels]." Furthermore, Dewinter et al. conclude that there is little chance that children with type 1 diabetes will exceed ADIs for steviol glycosides.

A. Food Uses as Addressed by JECFA, Merisant & Cargill

As part of its safety deliberations, JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). These estimates are presented in Table 8-1. Merisant also listed intended use levels of rebaudioside A for various food applications in their GRAS Notification (Table 8-2). Merisant utilized food consumption survey data from 2003-2004 National Health and Nutrition Examination Survey (NHANES) to determine the estimated daily intake from the proposed uses of rebaudioside A. On a per user basis, the mean and 90th precentile daily consumption levels of rebaudioside A were estimated as 2.0 and 4.7 mg per kg bw per day, respectively. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that, with a few minor exceptions, rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. Findings from the above-described different sources along with FSANZ estimates and the intake estimates are presented in Table 8-3.

B. Estimated Daily Intake

The very conservative consumer intake estimates provided by JECFA as shown in Table 8-1 were utilized to gauge the potential human exposures of rebaudioside A and steviol glycosides and in foods as reported in the US and in other countries. As rebaudioside A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly.

Table 8-1. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents

Food Type	Maximum Use Level Reported[a] (mg steviol glycosides/kg of food)	Maximum Use Level Calculated for rebaudioside A[b] (mg rebaudioside A/kg of food)	Maximum Use Level Calculated for rebaudioside A[b] (mg steviol equivalents/kg of food)
Desserts	500	250	83
Cold confectionery	500	250	83
Pickles	1000	500	167
Sweet corn	200	100	33
Biscuits	300	150	50
Beverages	500	250	83
Yogurt	500	250	83
Sauces	1000	500	167
Delicacies	1000	500	167
Bread	160	80	27

[a] Reproduced from WHO (2006).
[b] Calculated by Expert Panel assuming twice the sweetness intensity for rebaudioside A and three-fold difference in molecular weight between rebaudioside A and steviol.

Table 8-2. Proposed Uses & Levels of Rebaudioside A by Merisant[a]

Food Uses	Reb A (ppm)
Tabletop sweeteners	30,000[b]
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

[a] Merisant (2008)
[b] Reb A content of sachet prior to dilution and not representative of "as consumed."

Further consideration was given to anticipated human exposures as projected independently and with different approaches by JECFA (WHO, 2006), Merisant (2008), and Cargill (2008). As described below, the multiple approaches tended to converge to yield estimated daily intakes (EDIs) in the range of 1.3-4.7 mg per kg bw per day that, when compared to the acceptable daily intake (ADI), constitutes supporting information in the subject GRAS evaluation.

JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food. JECFA used the Global Environment Monitoring System (GEMS)/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg per kg bw per day with the African diet to 3.5 mg per kg bw per day with the European diet. Additionally, JECFA estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. The exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee are summarized in Table 8-4.

JECFA concluded that the replacement estimates were highly conservative---that is, the calculated dietary exposure overestimates likely consumption---and that true dietary intakes of steviol glycosides (as steviol) would probably be 20-30% of these values or 1.0-1.5 mg per kg bw per day on a steviol basis or 3.0-4.5 mg per kg bw per day for rebaudioside A based on the molecular weight adjustment. Similarly, FSANZ (2008) estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario, which resulted in estimated exposures of 0.3-1.0 mg per kg bw per day for the mean and 90th percentile consumer, or 0.5-1.5 mg per kg bw per day for rebaudioside A when making both the molecular weight and sweetness equivalency calculations. FSANZ examined consumption in other age groups and concluded that there were no safety concerns for children of any age. Merisant also calculated a dietary estimate for Reb A of 2.0 mg per kg bw per day for the average consumer and 4.7 mg per kg bw per day for a 90th percentile consumer. On a steviol equivalent basis, the Merisant estimates would be 0.7 and 1.6 mg per kg bw per day, respectively. In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3-3.4 mg per kg bw per day when calculated as Reb A (Renwick, 2008).

Table 8-3. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of EDI

Scenarios	EDI		
	As Steviol[a] (mg/kg bw/day)	As Rebaudioside A[b] (mg/kg bw/day)	Total Daily Intake[c] (mg/day)
JECFA			
100% Reb A replacement of sugars	5.0	7.5	450
20-30% Reb A replacement of sugars	1.0 - 1.5	1.5 - 2.3	90 - 140
FSANZ			
100% Reb A replacement of sugars	0.3 - 1.0	0.5 - 1.5	30 - 90
MERISANT			
		2.0 - 4.7[d]	120 - 282
CARGILL			
		1.3 - 3.4[d]	78 - 204

[a] Published values for mixed steviol glycosides consumption listed in this column were used for the calculation of Reb A consumption values appearing in next two columns.

[b] Estimates for Reb A consumption were calculated from JECFA and FSANZ estimates as steviol by multiplying by 3 to correct for the molecular weight of Reb A compared to steviol and by subsequently dividing by 2 because of the increased inherent sweetness of Reb A compared to the mixed steviol glycosides.

[c] Total daily intake figures were calculated for a 60 kg adult.

[d] Published values are shown for comparison purposes.

Table 8-4. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)

Estimate	Exposure (mg/kg bw/day)
GEMS/Food (International)[a]	1.3 -3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate[b]	3
US, Replacement Estimate[b]	5

[a] WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.

[b] These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

In October 2009, Cargill applied to FSANZ to increase the maximum usage levels of high purity steviol glycosides in the high-volume food categories of ice cream and various beverages. Cargill supported its application with increased usage levels by presenting market share analyses that

overestimate actual intake while remaining well below the generally accepted ADI. In December 2010, FSANZ recommended accepting the increased usage levels as requested since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved the Cargill application to increase the allowed maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg per kg and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).

On January 13, 2011, EFSA revised its dietary exposure assessment of steviol glycosides. For high consumers, revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg per kg bw (steviol equivalent). For European children aged 1-14 years, revised intake estimates ranged from 1.7 to 16.3 mg per kg bw per day, and for adults, the range was reported to be from 5.6 to 6.8 mg per kg bw per day (EFSA, 2011b).

Most recently, Roberts et al. (2016) suggested that a higher ADI is justified based on metabolic factors to reduce the 100X safety factor. A chemical-specific adjustment factor (CSAF), as defined by the WHO in 2005, was determined by comparative studies in rats and humans. A CSAF that is less than the standard 100X safety factor will result in an increase in the ADI, independent of the no observed adverse effect level (NOAEL). The authors determined that using a CSAF can justify an ADI value of 6-16 mg per kg bw per day for steviol glycosides, depending on whether area under the plasma-concentration time curve (AUC) or C_{max} data are used when considering the 1,000 mg per kg bw per day NOAEL (which is equivalent to 400 mg per kg bw per day of steviol) for stevioside reported by Toyoda et al. (1997).

There have been many scholarly estimates of potential dietary intake of replacement sweeteners---including steviol glycosides---that have been published (FSANZ, 2008; Renwick, 2008; WHO, 2003) or submitted to FDA (Merisant, 2008). In GRN 301, a simplified estimate was proposed to and accepted by FDA based on the estimates of exposure in “sucrose equivalents” (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg per kg bw per day for any population subgroup.

Appendix 9 Studies on Steviol Glycosides Preparations That Are Primarily Mixtures of Stevioside & Rebaudioside A

This appendix summarizes studies on stevioside or stevia extracts that were identified compositionally as predominantly stevioside. In some of the published literature, the terms stevia, stevioside, and stevia glycoside are used interchangeably. However, wherever possible, an attempt has been made to identify the specific substance studied.

1. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

Several studies in rats (Wingard Jr et al., 1980; Nakayama et al., 1986; Koyama et al., 2003b) and other animal models, including chickens (Geuns et al., 2003b), hamsters (Hutapea et al., 1999), and pigs (Geuns et al., 2003a), indicate that stevioside is not readily absorbed from the gastrointestinal (GI) tract. Available evidence from *in vitro* metabolism studies suggests that bacteria in the colon of rats and humans can transform various stevia glycosides into steviol (Gardana et al., 2003). Steviol was shown to be more readily transported with *in vitro* intestinal preparations than various steviosides (Geuns et al., 2003a; Koyama et al., 2003b). Slow absorption of steviol was indicated by detection in the plasma of rats given oral stevioside (Wang et al., 2004). However, Sung (2002) did not detect plasma steviol following oral administration of steviosides to rats. In studies with human and rat liver extracts, Koyama et al. (2003b) demonstrated that steviol can be converted to various glucuronides. Excretion of metabolites of stevioside after oral doses has been shown in urine and feces in rats (Sung, 2002) and hamsters (Hutapea et al., 1999). Oral doses in pigs led to the detection of metabolites in feces but not in urine (Geuns et al., 2003a).

Koyama et al. (2003b) published an *in vitro* study in which α-glucosylated steviol glycosides were degraded by fecal microflora to steviol glycosides. These are subsequently hydrolyzed to the aglycone, steviol, demonstrating that the metabolic fate of α-glucosylated steviol glycosides follows that of non-modified steviol glycosides. Due to the similarities in metabolic fate, the safety of α-glucosylated steviol glycosides can be established based on studies conducted with non-modified steviol glycosides. Furthermore, as individual steviol glycosides show similar pharmacokinetics in the rat and humans, the results of toxicology studies on individual steviol glycosides are applicable to the safety of steviol glycosides in general.

In a human study with 10 healthy subjects, Geuns et al. (2006) measured blood, urine, and fecal metabolites in subjects who received 250 mg of purified stevioside (>97%) three times a day for 3 days. Urine was collected for 24 hours on day 3, and blood and fecal samples were also taken on day 3. Free steviol was detected in feces but not in blood or urine. Steviol glucuronide was detected in blood, urine, and feces. Approximately 76% of the total steviol equivalents dosed were recovered in urine and feces. Based on these measurements, the authors concluded that there was complete conversion of stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide.

Renwick and Tarka (2008) reviewed studies on microbial hydrolysis of steviol glycosides. The reviewers concluded that stevioside and Reb A are not absorbed directly, and both are converted to steviol by gut microbiota in rats and in humans. This hydrolysis occurs more slowly for Reb A than for stevioside. Studies have shown that steviol-16,17-epoxide is not a microbial metabolite.

2. Acute Toxicity Studies

The oral LD_{50} studies of stevioside (purity, 96%) following administration of a single dose to rodents are summarized in Table 9-1. No lethality was noted within 14 days after the administration, and no clinical signs of toxicity, or morphological or histopathological changes were found, indicating that stevioside is relatively harmless.

Table 9-1. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents

SPECIES	SEX	LD_{50} (G/KG BW)	REFERENCE
Mouse	Male and Female	>15	Toskulkac et al. (1997)
Mouse	Male	> 2	Medon et al. (1982)
Rat	Male and Female	>15	Toskulkac et al. (1997)
Hamster	Male and Female	>15	Toskulkac et al. (1997)

3. Subchronic Toxicity Studies

In five published studies, subchronic toxicity of stevioside was investigated in rats following oral administration. In addition, a reproduction study in hamsters included subchronic phases on the F_0, F_1, and F_2 generations. These studies are summarized in Table 9-2. One of these studies was particularly important because it served as a range-finding study for two subsequent chronic studies. In this 13-week toxicity study, Fischer 344 rats (10 per sex per group) were given doses of 0, 0.31, 0.62, 1.25, 2.5, or 5% in the diet (equivalent to 160, 310, 630, 1,300, and 2,500 mg per kg bw per day) to determine the appropriate doses for a two-year carcinogenicity study. None of the animals died during the administration period, and there was no difference in body weight gain between the control and treated groups during administration or in food consumption in the latter part of the study. The activity of lactic dehydrogenase and the incidence of single-cell necrosis in the liver were increased in all groups of treated males. The authors considered these effects to be nonspecific, because of the lack of a clear dose-response relationship, the relatively low severity, and their limitation to males. Other statistically significant differences in hematological and biochemical parameters were also considered to be of minor toxicological significance. The authors concluded that a concentration of 5% in the diet was a suitable maximum tolerable dose of stevioside for a two-year study in rats (Aze et al., 1990).

In earlier 3-month rat studies reviewed by Geuns et al. (2003a)---the sample purity, doses, and strain of rat were not reported---a no effect level was determined to be in excess of 2,500 mg per

kg bw per day and 7% of the diet, apparently due to lack of effects at the highest dose tested in both studies (Akashi and Yokoyama, 1975).

In a published exploratory subchronic toxicity study, Awney et al. (2011) investigated the effects of 97% pure stevioside on body weight, organ relative weight, hematological and biochemical parameters, and enzyme activities in Sprague Dawley rats. In this 12-week toxicity study, groups of male rats (8 per group) were given drinking water containing stevioside. The groups were assigned to drink distilled water (control), low-dose stevioside solution (15 mg per kg per day), high-dose stevioside solution (1,500 mg per kg per day), or low-dose stevioside (15 mg per kg per day) plus inulin solution for 12 weeks as the sole source of liquid. Fluid intake was recorded daily, and levels of test articles were adjusted weekly to achieve the appropriate target concentration. Low-dose stevioside (15 mg per kg bw per day) administration, with or without inulin, for 12 weeks did not reveal any adverse effects on body weight, organs relative weight, hematological and biochemical parameters, or enzyme activities. However, treatment with high-dose stevioside was reported to cause significant changes in several investigated toxicological parameters. Among the hematological parameters, significant changes were noted in all except white blood cells (WBCs), red blood cells (RBCs), and packed cell volume (PCV%), and in all clinical chemistry parameters except proteins, total lipids, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). These data support the no observed effect level (NOEL) of 15 mg per kg per day. However, critical review of the publication reveals that the study was poorly designed and implemented. Design deficiencies include: insufficient numbers of animals; group-housing with the potential for stress-related changes; unreliable access to steviol *via* drinking water, resulting in suspect dosing calculations in group-housed cages; no indication of fasting prior to blood collection, which affects many chemistry and hematological values; no urine collection; and no histopathological evaluations for confirmation of findings beyond the controls. In addition to these study design deficiencies, the report fails to adequately present mean or individual organ weight data and, in general, there appears to be inadequate comparison of study findings against laboratory historical control data. Any one of these oversights could have adversely affected the results and/or interpretation of the hematological and chemistry data.

In addition to the above-described parameters, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased (Awney et al., 2011). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells. This enzyme was not measured in any previous steviol glycosides studies nor has it been adequately vetted for application in toxicological studies. These investigators did not identify the specific TRAP isomer measured, the methodology employed, the handling of the samples, or any historical data on TRAP levels. The significance and relevance of this poorly documented toxicological endpoint, which lacks histopathological confirmation, does not appear to have a distinct role in determining the toxicological profile of a material in a test animal. The data presented by Awney et al. (2011) are probably not representative of changes due to the subchronic dietary administration of steviol glycosides because of overall inadequate study design and reliance on the findings of the untested enzyme TRAP. The preponderance of the data from

several well-designed studies on steviol glycosides suggest that differences noted in hematological and chemistry data are probably random, nonspecific, and not toxicologically significant.

Critical reviews of the publication by Carakostas (2012) and Waddell (2011) revealed a poor study design that included: insufficient numbers of animals; group-housing with the potential for stress-related changes; unreliable access to steviol *via* drinking water resulting in suspect dosing calculations in group-housed cages; no indication of fasting prior to blood collection, which affects many chemistry and hematological values; no urine collection; and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the report did not adequately describe mean or individual organ weight data and lacked comparison of study findings against laboratory historical control data.

Table 9-2. Summary of Subchronic Studies on Stevioside

Study	Animal Model/ Group Size	Test Material/ Sample Purity	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Aze et al. (1990)[a]	F344 rat/ 10 females & 10 males in each of 6 groups	Stevioside/ Not reported	0, 0.31, 0.62, 1.25, 2.5, 5% in diet/day/13 weeks	Not reported	No effects observed on mortality, body weight or food consumption. Clinical chemistry investigation revealed increased LDH levels & histopathological investigation indicated increased incidence of single-cell liver necrosis in all male treated groups, but no clear dose-response relationship. Investigators did not consider these changes to be treatment related due to small magnitude & low severity of changes, the lack of clear dose response relationship & limitation to males only. Organ weights, urine chemistry & gross necropsy not discussed. Authors concluded that 5% stevioside in diet is tolerable dose for 2-year study.
Mitsuhashi (1976)[b]	Rat (strain not reported)	Stevioside/ Not reported	Dietary concentrations up to 7%/day/ 3 months	Not reported	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy & histopathology not discussed.
Akashi and Yokoyama (1975)[b]	Rat (strain not reported)	Stevioside/ Not reported	Oral doses up to 2,500 mg/kg bw/day/3 months	2,500	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy & histopathology not discussed.
Awney et al. (2011)	Sprague Dawley rats	Stevioside 97%	Drinking water (15, 1,500 mg/kg bw /day/12 weeks)	15	Treatment with high dose stevioside caused significant changes in several investigated toxicological parameters. Among hematological parameters, significant changes noted in all except WBCs, RBCs & PCV% & in all clinical chemistry parameters except proteins, total lipids, ALT and AST.

[a] Abstract only [b] As reported by Geuns et al. (2003a)

4. Chronic Toxicity Studies

Chronic effects of stevioside have been studied in three separate studies (Table 9-3). No treatment-related increase in tumor incidence was seen in any of these studies. In the most recent and well-documented study for which additional study details were presented to JECFA in 2006 (WHO, 2006), the apparent no observed adverse effect level (NOAEL) in F344 rats was the dietary level of 2.5% [test sample purity 96%, Toyoda et al. (1997)]. At 5% of the diet, statistically significant decreases in body weight, percent survival, and kidney weight were noted. The authors attributed these effects to various factors. The decrease in body weight was attributed to an inhibition of glucose utilization. The decrease in survival seemed to have been caused by an unusual late onset of large granular lymphocyte leukemia in high dose males. The authors reported that this tumor is rather common in F344 rats and that the overall incidence in male rats was actually within the historical control range experienced in the laboratory where studies were conducted. According to the authors, the decrease in kidney weight was probably due to a decrease in chronic inflammation found in the histopathological examination relative to control animals.

Table 9-3. Summary of Chronic Toxicity Studies on Stevioside

Study	Animal Model/ Group Size	Test Material/ Sample Purity	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Toyoda et al. (1997)	F344 rat/ 50 per sex per group	95.6% Stevioside	*Ad libitum* 0,2.5, 5% of diet/~24 months (104 weeks)	Author did not assign a NOAEL. (Mid-dose calculates to 970 in males; JECFA, 2006)	Significant decrease in survival rates in males receiving 5%. General condition, body weight, food intake, mortality, hematological, histopathological & organ weights observed or measured. Body weight gains dose-dependently decreased in both sexes. Kidney weights significantly lower in 5% males & ovary, kidney, & brain weights significantly increased in 5% females. Tumors & non-neoplastic lesions found in all groups & not correlated to treatment. Conclusion--stevioside is not carcinogenic under these experimental conditions.
Xili et al. (1992)[a]	Wistar rat/ 45 per sex per group	85% Stevioside	0, 0.2, 0.6, 1.2 % of diet/24 months	794 (high dose)	After 6, 12 & 24 months, 5 rats from each group sacrificed for analysis. No effects observed on growth, food utilization, general appearance, mortality, or lifespan. No changes in hematological, urinary, or clinical biochemical values. Histopathological analysis showed that the neoplastic and non-neoplastic lesions unrelated to level of stevioside in diet.
Yamada et al. (1985)	F344 rat/ 70 per sex per group, 30 per sex per group in low-dose	95.2% Steviol glycosides (75% stevioside; 16% Reb A)	0.1, 0.3, 1% of diet/22 months for males, 24 months for females	550 (high dose)	At 6 &12 months, 10 males & 10 females sacrificed for analysis. General behavior, growth & mortality were same among groups throughout experiment. At 6 months, protein urea significantly increased in females, & blood glucose increased in both sexes, although urinary glucose not detected. Weights of liver, kidney, heart, prostate & testes increased in males at 6 months, &weight of ovaries decreased in females in dose-dependent manner.

Study	Animal Model/ Group size	Test Material/ Sample Purity	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
					Histopathological examination showed differences in various organs at 6 months that were unrelated to stevioside dose. These differences not found at 12 months. Authors concluded that there were no significant changes after 2 years.

[a] Only abstract available

5. Reproductive & Developmental Toxicity Studies

The use of *S. rebaudiana* as an oral contraceptive has been reported by Indians in Paraguay (Planas and Kuć, 1968; Schvartaman et al., 1977). In experimental studies in rats, crude stevia leaf extract has been shown to inhibit fertility (Planas and Kuć, 1968). Reproductive toxicity studies have been conducted with orally administered purified stevioside. No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses up to 2,500 mg per kg per day (Yodyingyuad and Bunyawong, 1991). There was an absence of statistically significant effects at doses up to 3% [equivalent to 3,000 mg per kg bw per day; sample purity 96%; Mori et al. (1981)]. Similar results were observed in an additional rat study that was reviewed by Geuns et al. (2003a) where limited information is available in English (Usami et al., 1994).

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1,000 mg per kg bw per day (only 12 animals at the highest dose) by gavage in corn oil on days 6 - 10 of gestation. A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12, respectively) were observed at the three highest doses, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). However, no dose-dependent teratogenic effects were seen. The NOEL was 250 mg per kg bw per day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

No effect on pregnancy or developmental parameters was observed in Swiss albino mice with stevioside or aqueous stevia extract at doses up to 800 mg per kg bw per day in female mice (Kumar and Oommen, 2008). Further details on these studies to the extent available are presented in Table 9-4.

Table 9-4. Summary of Reproductive Toxicity Studies on Steviol Glycosides

Study	Animal Model/ Group Size	Test Sample Purity Stevioside (unless Otherwise Noted)	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results & Remarks
Kumar and Oommen (2008)	Swiss albino mice/ 4 groups of 5 females	Not reported	500 & 800 mg/kg bw/day/15 days	800	Stevioside & stevia extract (purity & composition not reported) did not have any effect on reproductive parameters in mice when administered to female mice before or during pregnancy. No changes seen in number of implantations or uterine resorptions. No gross anatomical or histopathologic effects seen in 16-day embryos.
Usami et al. (1994)[a]	Wistar rat/4 groups of 25 or 26 pregnant rats	95.6%[b]	0, 250, 500, 1,000 mg/kg bw/day/10 days	1,000	Pregnant rats given doses of stevioside by gavage once/day on days 6-15 of gestation & were sacrificed on day 20 of gestation. Fetuses examined for malformations in addition to maternal & fetal body weight, number of live fetuses, sex distribution & numbers of resorptions or dead fetuses. No treatment-related effects observed. Authors concluded that orally administered stevioside not teratogenic in rats.
Yodyingyuad and Bunyawong (1991)	Hamster/ 10 male, 10 female per group (40 total)	90%	0, 500, 1,000, 2,500 mg/kg bw/day/ duration unclear/ 3 months	2,500	Males from each group mated to females from respective dose group. Each female allowed to bear 3 litters during course of experiment. Stevioside had no effect on pregnancies of females at any dose. The F_1 & F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents); showed normal growth & fertility. Histological examination showed no effect on reproductive organs at any dose.
Oliveira-Filho et al. (1989)[a]	Rat/ number not reported	Not reported (Dried Stevia Leaves)	0 or 0.67 g dried leaves/mL, 2 mL twice per day/ 60 days	Not reported	Prepubertal rats (25-30 days old) tested for glycemia; serum concentrations of thyroxine; tri-iodothyroxine; available binding sites in thyroid hormone-binding proteins; binding of ^{3}H-methyltrienolone (a specific ligand of androgen receptors) to prostate cytosol; zinc content of prostate, testis, submandibular salivary gland, & pancreas; water content of testes & prostate; body-weight gain; & final weights of testes, prostate, seminal vesicle, submandibular salivary gland& adrenal. Only difference due to treatment was seminal vesicle weight, which fell to 60% compared to control.
Mori et al. (1981)	Rat/11 male, 11 female per group (44 total)	96%	0, 0.15, 0.75 or 3 % of feed/60 days	2,000	Males given stevioside dose in diet for 60 days before & during mating with females who received same diet (as mated male) 14 days before mating & 7 days during gestation. No effect due to treatment on fertility or mating performance& no effect of fetal development. Rats of each sex had slightly decreased body weight gain at highest dose with non-significant increase in number of dead & resorbed fetuses at highest dose.

Study	Animal Model/ Group size	Test Sample Purity Stevioside (UNLESS Otherwise Noted)	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results & Remarks
Planas and Kuć (1968)	Rat/14 per group (28 total)	Not reported (Crude stevia extract)	0 or 5% Crude stevia extract /18 days	Not reported	Extract given orally to adult female rats for 12 days, who were mated with untreated males during last 6 days. Fertility reduced to 21% of fertility in control rats & remained reduced in a 50-60 day recovery. Histological examination, weights of organs, blood analysis, urine chemistry and & necropsy not discussed.

[a] Only abstract available [b] As reported by EuropeanCommission (1999b)

6. Mutagenicity & Genotoxicity Studies

In a series of studies, mutagenic and genotoxic effects of various stevia extracts and various preparations of stevioside were investigated. These studies are summarized in Table 9-5. All studies were negative with the exception of a comet assay done in rats (Nunes et al., 2007). The methodology used in this study, and the resulting conclusions, have been questioned by Geuns (2007), Williams (2007), and Brusick (2008), and responded to by the authors (Nunes et al., 2007b; Nunes et al., 2007c)

Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern has been expressed in two publications (Brahmachari et al., 2011; Tandel, 2011) in which the authors concluded that additional testing is necessary to adequately address the genotoxicity profile (Urban et al., 2013). The review aimed to address this matter by evaluating the specific genotoxicity studies of concern, while evaluating the adequacy of the database that includes more recent genotoxicity data not noted in these publications. The results of this literature review showed that the current database of *in vitro* and *in vivo* studies for steviol glycosides is robust and does not indicate that either stevioside or rebaudioside A are genotoxic. This finding, combined with lack of carcinogenic activity in several rat bioassays, establishes the safety of all steviol glycosides with respect to their genotoxic/carcinogenic potential.

Table 9-5. Mutagenicity & Genotoxicity Studies on Stevia Extracts & Stevioside

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
In Vitro						
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate[a] 1 mg/plate[b]	Negative	Matsui et al. (1996)

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	99	50 mg/plate	Negative[c]	Suttajit et al. (1993)
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	NS	50 mg/plate	Negative	Klongpanichpak et al. (1997)
Forward mutation	*S. typhimurium* TM677	Stevioside	83	10 mg/plate	Negative[c]	Matsui et al. (1996)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	10 mg/plate	Negative[c]	Pezzuto et al. (1985)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	Not specified	Negative[c]	Medon et al. (1982)
Gene mutation	Mouse lymphoma L5178Y cells, TK$^-$ locus	Stevioside	NS	5 mg/mL	Negative[c,d]	Oh et al. (1999)
Gene mutation (umu)	*S. typhimurium* TA1535/pSK1002	Stevioside	83	5 mg/plate	Negative[c]	Matsui et al. (1996)
Gene mutation	*B. subtilis* H17 rec+, M45 rec-	Stevioside	83	10 mg/disk	Negative[c]	Matsui et al. (1996)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	83	8 mg/mL 12 mg/mL	Negative	Matsui et al. (1996)
Chromosomal aberration	Human lymphocytes	Stevioside	NS	10 mg/mL	Negative	Suttajit et al. (1993)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	85	12 mg/mL	Negative[a]	Ishidate et al. (1984)
In Vivo						
DNA damage (comet assay)	Wistar rats; liver, brain and spleen	Stevioside	88.62	4 mg/L (estimated to be 80 - 500 mg/kg bw/day) in drinking water for 45 days	Positive in all tissues examined, most notably in liver	Nunes et al. (2007)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52; Reb A, 22	250 – 2,000 mg/kg bw	Negative[e]	Sekihashi et al. (2002)
DNA damage (comet assay)	Male ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	Stevia	NS	2,000 mg/kg bw	Negative[e]	Sasaki et al. (2002)
Micronucleus formation	ddY mouse bone marrow and regenerating liver	Stevioside	NS	62.5 - 250 mg/kg bw	Negative	Oh et al. (1999)
Mutation	*D. melanogaster* Muller 5 strain	Stevioside	NS	2% in feed	Negative	Kerr et al. (1983)

NS = Not specified [a] Without metabolic activation [b] As calculated by Williams (2007) [c] With and without metabolic activation (source not specified in original monograph) [d] Inadequate detail available [e] Sacrificed at 3 hours and 24 hours

7. Clinical Studies & Other Reports in Humans

In several studies, pharmacological and biochemical effects of crude extracts of stevia leaves and purified steviol glycosides have been investigated. The effects noted included glucose uptake, insulin secretion, and blood pressure (Geuns et al., 2003a). In South America, stevioside is used as a treatment for type 2 diabetes. These effects were key concerns for JECFA. In 2006, JECFA summarized the available clinical studies of stevioside and further studies were recommended

(WHO, 2006). Subsequently, several studies were conducted, and in 2009, JECFA reviewed these new studies (WHO, 2009). JECFA's summaries of the key studies are included below.

a. Studies Summarized in 2006

In a study by Curi et al. (1986), aqueous extracts of 5 grams of *S. rebaudiana* leaves were administered to 16 volunteers at 6 hour intervals for three days, and glucose tolerance tests were performed before and after the administration. Another six volunteers were given an aqueous solution of arabinose in order to eliminate possible effects of stress. The extract increased glucose tolerance and significantly decreased plasma glucose concentrations during the test and after overnight fasting in all volunteers.

In a multi-center randomized, double-blind, placebo-controlled trial of hypertensive Chinese men and women (aged 28–75 years), 60 patients were given capsules containing 250 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 750 mg of stevioside per day [equivalent to 11 mg per kg bw per day as calculated by FSANZ (2008)] and followed up at monthly intervals for one year. Forty-six patients were given a placebo. After 3 months, systolic and diastolic blood pressure in men and women receiving stevioside decreased significantly, and the effect persisted over the year. Blood biochemistry parameters, including lipids and glucose, showed no significant changes. Three patients receiving stevioside and one receiving the placebo withdrew from the study as a result of side effects (nausea, abdominal fullness, dizziness). In addition, four patients receiving stevioside experienced abdominal fullness, muscle tenderness, nausea, and asthenia within the first week of treatment. These effects subsequently resolved, and the patients remained in the study (Chan et al., 2000).

In a follow-up multi-center randomized, double-blind, placebo-controlled trial was conducted in hypertensive Chinese men and women (aged 20–75 years), 85 patients were given capsules containing 500 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 1,500 mg of stevioside per day [equivalent to 21 mg per kg bw per day, as calculated by FSANZ (2008)]. Eighty-nine patients were given a placebo. During the course of study, three patients in each group withdrew. There were no significant changes in body mass index or blood biochemistry parameters throughout the study. In the group receiving stevioside, mean systolic and diastolic blood pressures were significantly decreased compared with the baseline, commencing from about 1 week after the start of treatment. After 2 years, 6 out of 52 patients (11.5%) in the group receiving stevioside had left ventricular hypertrophy compared with 17 of 50 patients (34%) in the group receiving the placebo ($p < 0.001$). Eight patients in each group reported minor side effects (nausea, dizziness and asthenia), which led two patients in each group to withdraw from the study. Four patients in the group receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved and the patients remained in the study (Hsieh et al., 2003).

In a randomized, double-blind trial designed, 48 hyperlipidemic volunteers were recruited to investigate the hypolipidemic and hepatotoxic potential of steviol glycoside extract. The extract

used in this study was a product containing stevioside (73 ± 2%), rebaudioside A (24 ± 2%), and other plant polysaccharides (3%). The subjects were given two capsules, each containing 50 mg of steviol glycoside extract or placebo, twice daily (i.e., 200 mg per day, equivalent to 3.3 mg per kg bw per day assuming an average body weight of 60 kg), for 3 months. One subject from placebo group and three from treatment group failed to complete the study for personal reasons, not related to adverse reactions. At the end of the study, both groups showed decreased serum concentrations of total cholesterol and of low-density lipoproteins. Analyses of serum concentrations of triglycerides, liver-derived enzymes, and glucose indicated no adverse effects. The authors questioned the subjects' compliance with the dosing regimen, in view of the similarity of effect between treatment and placebo (Anonymous, 2004). In a follow-up study, 12 patients were given steviol glycosides extract in incremental doses of 3.25, 7.5, and 15 mg per kg bw per day for 30 days per dose. Preliminary results indicated no adverse responses in blood and urine biochemical parameters (Anonymous, 2004).

In a paired cross-over study, 12 patients with type 2 diabetes were given either 1 gram of stevioside (stevioside, 91%; other stevia glycosides, 9%) or 1 gram of maize starch (control group), which was taken with a standard carbohydrate-rich test meal. Blood samples were drawn at 30 minutes before, and for 240 minutes after, ingestion of the test meal. Stevioside reduced postprandial blood glucose concentrations by an average of 18% and increased the insulinogenic index by an average of 40%, indicating beneficial effects on glucose metabolism. Insulin secretion was not significantly increased. No hypoglycemic or adverse effects were reported by the patients or observed by the investigators. Systolic and diastolic blood pressure was not altered by stevioside administration (Gregersen et al., 2004).

b. Studies Summarized in 2009

In a short-term study of stevioside in healthy subjects, 4 male and 5 female healthy volunteers (aged 21–29 years) were provided with capsules containing 250 mg stevioside (97% purity) to be consumed 3 times per day for 3 days (Temme et al., 2004). Doses, expressed as steviol, were 288 mg per day, or 4.4 mg per kg bw per day for females and 3.9 mg per kg bw per day for males. Twenty-four-hour urine samples were taken before dosing on day 1 and after dosing on day 3. Fasting blood samples were taken before dosing on day 1, and six samples were taken at different time points on day 3 after dosing. Fasting blood pressure measurements were taken before the first capsule and at six different time intervals after the first dose. Urine was analyzed for creatinine, sodium, potassium, calcium, and urea. Blood was analyzed for plasma glucose, plasma insulin, alkaline phosphatase, alanine transaminase (ALT), glutamic-pyruvate transaminase (GPT), creatine kinase, and lactate dehydrogenase. The clinical analyses of blood, blood pressure, and urine showed no differences between samples taken before or after dosing.

In an unpublished double-blind, placebo-controlled trial study reviewed at the 68th JECFA meeting, 250 mg of a product containing 91.7% total steviol glycosides, including 64.5% stevioside and 18.9% rebaudioside A, was administered to groups of type 1 (*n* = 8) and type 2 diabetics (*n* = 15), and non-diabetics (*n* = 15), 3 times daily for 3 months. Control groups with the same number of

subjects received a placebo. After 3 months, there were no significant changes in systolic or diastolic blood pressure, glycated hemoglobin (HbA1c), blood lipids, or renal or hepatic function. No adverse effects were reported. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Barriocanal et al., 2006; Barriocanal et al., 2008). The Committee previously noted that this product did not meet the proposed specification of "not less than 95% steviol glycosides" and that the study was conducted in a small number of subjects.

In a follow-up study, Barriocanal et al. (2008) evaluated the effects of steviol glycosides on blood glucose and blood pressure (BP) for three months in subjects with type 1 diabetes, subjects with type 2 diabetes, and subjects without diabetes and with normal/low-normal BP levels. Patients in each group received either 250 mg total dissolved solids (tds) steviol glycoside, stevioside, or placebo treatment. The purity of the steviol glycosides was ≥ 92%. Three months of follow up revealed no changes in systolic BP, diastolic BP, glucose, or glycated hemoglobin from baseline. In placebo type 1 diabetics, there was a significant difference in systolic BP and glucose. There were no adverse effects observed in either treatment group, and the authors concluded that oral steviol glycosides are well-tolerated and have no pharmacological effect.

A study of antihypertensive effects was conducted in previously untreated mild hypertensive patients with crude stevioside obtained from the leaves of *S. rebaudiana*. Patients with essential hypertension were subjected to a placebo phase for 4 weeks and then received either capsules containing placebo for 24 weeks or crude stevioside at consecutive doses of 3.75 mg per kg bw per day (7 weeks), 7.5 mg per kg bw per day (11 weeks) and 15 mg per kg bw per day (6 weeks). Comparison of patients receiving stevioside with those on placebo showed neither antihypertensive nor adverse effects of stevioside. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Ferri et al., 2006). The product in this study also did not meet the proposed specification.

A placebo-controlled double-blind trial was carried out in 49 hyperlipidemic patients (aged 20–70 years, number of males and females not supplied) not undergoing treatment. The study was approved by the local ethics committee and complied with the principles of the Declaration of Helsinki. Individuals were divided into two groups, with 24 subjects receiving placebo capsules and 25 receiving capsules containing a dose of 50 mg steviol glycosides (70% stevioside, 20% Rebaudioside A), equivalent to 1.04 mg steviol per kg bw per day, using the mean body weight of the treatment group, 72.7 kg. Two capsules were taken before lunch, and two before dinner, each day for 90 days. Six subjects withdrew from the study, four in the placebo group and two in the test group. Self-reported adverse reactions were recorded, and fasting blood samples were taken at the end of the study and analyzed for alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglycerides. No effects of treatment on ALT, AST, or GGT were found. Decreases in the total cholesterol and LDL were observed in both the stevioside group and the placebo group, which were not treatment related. No

adverse effects were observed (Silva et al., 2006). The Committee noted at its 68th meeting that the product used in this study did not meet the proposed specification.

In a long-term, randomized, double blinded, placebo-controlled study, Jeppesen et al. (2006) investigated the efficacy and tolerability of oral stevioside in patients with type 2 diabetes. In this study, 55 subjects received 500 mg stevioside (purity unspecified), or placebo (maize starch), 3 times daily for 3 months. Compared with the placebo, stevioside did not reduce the incremental area under the glucose response curve and maintained the insulin response, HbA1c, and fasting blood glucose levels. HbA1c is an indicator of mean glucose levels and is used in identifying effects on the control of diabetes. No differences in lipids or blood pressure were observed. It is not clear whether this study was approved by the local ethics committee or met the requirements of the Declaration of Helsinki (Jeppesen et al., 2006).

Appendix 10 Summary of the Regulatory History of Steviol Glycosides

1. History of Traditional Medicinal and Human Food Use

Stevia has been used as a traditional medicine and sweetener by native Guarani tribes for centuries (Esen, 2016; Gerwig et al., 2016; Brusick, 2008; Brandle et al., 1998). Hawke (2003) reported that stevia is commonly used as a treatment for type 2 diabetes in South America**.** However, for its therapeutic effects, elevated doses in the range of 1 gram per person per day or more were reported to be necessary (Gregersen et al., 2004).

For about 30 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners (Raintree, 2012). It was previously reported that 40% of the artificial sweetener market in Japan had been stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Use of steviol glycosides as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand, and as a natural health product in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated that sales of stevia in the US reached $45 million (Newsday, 2006).

More recent reports of consumption figures for stevia reveal pronounced increases in global consumption. Worldwide, Zenith International estimates stevia sales of 3,500 metric tons in 2010, which represents a 27% increase over 2009 figures. The market value is estimated to have increased to $285 million (Zenith, 2011). In 2013, worldwide sales of stevia was reported to reach 4,100 tons which represents a 6.5% increase over 2011 figures, and this corresponds to an overall market value of $304 million (Zenith, 2013).

In October 2014, Zenith International reported that worldwide stevia sales were on course to increase 14% to 4,670 tons, associated with a market value of $336 million. Furthermore, it has been projected that the total market for stevia in 2017 will be 7,150 tons with an associated market value of $578 million (Zenith, 2014).

NewHope360 reported that the global market for stevia in 2014 was $347 million, and that is expected to increase to $565.2 million by 2020. In addition, consumption is expected to increase from 2014 levels of 5,100.6 tons to 8,506.9 tons by 2020 (NewHope360, 2015).

More recently, Nutritional Outlook reported that Mintel data indicated a 48% increase in stevia-containing products over the last five years (Decker and Prince, 2018).

2. Summary of Regulatory History of Enzyme Modified Steviol Glycosides

Stevia-derived sweeteners are permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, these sweeteners have received food usage approvals in Mexico, Australia, New Zealand, Switzerland, France, Peru,

Uruguay, Colombia, Senegal, Russia, Malaysia, Turkey, Taiwan, Thailand, Israel, Canada, and Hong Kong (EFSA, 2010; Watson, 2010; Health Canada, 2012). In the US, steviol glycosides have been used as a dietary supplement since 1995 (Geuns et al., 2003a).

a. U.S. Regulatory History

Based on available information from FDA's GRAS Notice Inventory website (FDA, 2018) as of December 11, 2018, FDA has issued 56 "no questions" letters on GRAS notices on rebaudioside A, rebaudioside D, rebaudioside M, or steviol glycosides, including those undergoing enzyme treatment. A summary of these filings is presented in Table 10-1.

Table 10-1. FDA's GRAS Notice Inventory on Various Steviol Glycosides Preparations[a,c]

COMPANY	FDA GRAS IDENTIFIER	MATERIAL IDENTITY	INTENDED FOOD USES
1. Merisant	GRN 252	High-Purity Reb A ≥95%	Variety of food categories & table top sweetener
2. Cargill Inc.	GRN 253	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat & poultry products
3. McNeil Nutritionals LLC	GRN 275	Purified Steviol Glycosides – Reb A Principal Component	Table top sweetener
4. Blue California	GRN 278	High-Purity Reb A ≥97%	General-purpose & table top sweetener
5. Sweet Green Fields LLC	GRN 282	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat & poultry products
6. Wisdom Natural Brands	GRN 287	Purified Steviol Glycosides >95% - Reb A and Stevioside Principal Component	General-purpose sweetener, excluding meat, poultry products & infant formulas
7. Sunwin USA LLC & WILD Flavors	GRN 303	High-Purity Reb A ≥95%/ ≥98%	General-purpose sweetener, excluding meat, poultry products & infant formulas
8. Sunwin USA LLC & WILD Flavors	GRN 304	Purified Steviol Glycosides >95% - Reb A and Stevioside Principal Component	General-purpose sweetener, excluding meat, poultry products & infant formulas
9. Pyure Brands, LLC	GRN 318	High-Purity Reb A 95%/ 98%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
10. PureCircle USA Inc	GRN 323	Purified Steviol Glycosides – Reb A Principal Component	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
11. GLG Life Tech Ltd[c]	GRN 329	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat & poultry products
12. NOW Foods	GRN 337	Enzyme Modified Steviol Glycosides Preparation (EMSGP)	General-purpose sweetener in foods, excluding meat & poultry products, at levels determined by good manufacturing practices
13. GLG Life Tech Ltd[c]	GRN 348	High-Purity Stevioside ≥95%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
14. GLG Life Tech Ltd[c]	GRN 349	High-Purity Steviol Glycosides ≥97%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas

COMPANY	FDA GRAS IDENTIFIER	MATERIAL IDENTITY	INTENDED FOOD USES
15. Guilin Layn Natural Ingredients, Corp.	GRN 354	High-Purity Reb A ≥97%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
16. BrazTek International Inc.	GRN 365	Purified Reb A	General-purpose sweetener, excluding meat & poultry products
17. Sinochem Qingdao Co. Ltd.	GRN 367	High-Purity Steviol Glycosides ≥95%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
18. Shanghai Freemen Americas LLC	GRN 369	Purified Reb A	General-purpose sweetener, excluding meat & poultry products
19. Toyo Sugar Refining Co., Ltd. & Nippon Paper Chemicals Co., Ltd.	GRN 375	Enzyme Modified Steviol Glycosides	General-purpose sweetener in foods, excluding meat and poultry products, at levels determined by good manufacturing practices
20. GLG Life Tech Ltd[b]	GRN 380	Purified Reb A	General purpose & table top sweetener, excluding meat & poultry products
21. Chengdu Wagott Pharmaceutical	GRN 388	Purified Reb A	General purpose & table top sweetener, excluding meat & poultry products
22. Chengdu Wagott Pharmaceutical	GRN 389	Steviol Glycosides with Stevioside as the Principal Component	General purpose & table top sweetener, excluding meat & poultry products
23. Daepyung Co., Ltd.	GRN 393	Purified Reb A	General purpose & table top sweetener, excluding meat & poultry products
24. Daepyung Co., Ltd.	GRN 395	Steviol Glycosides with Reb A and Stevioside as the Principal Components	General purpose & table top sweetener, excluding meat & poultry products
25. MiniStar International, Inc.	GRN 418	Purified Reb A	General-purpose sweetener, excluding meat, poultry products & infant formulas.
26. Daepyung Co., Ltd.	GRN 448	Enzyme Modified Steviol Glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas.
27. Daepyung Co., Ltd.	GRN 452	Enzyme Modified Steviol Glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas.
28. PureCircle USA, Inc.	GRN 456	High-Purity Reb D ≥95%	General-purpose sweetener, excluding meat, poultry products & infant formulas.
29. Almendra, Ltd.	GRN 461	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat, poultry products & infant formulas.
30. Qufu Xiangzhou Stevia Products Co., Ltd.	GRN 467	High-Purity Reb A ≥98%	General-purpose sweetener, excluding meat, poultry products & infant formulas.
31. PureCircle USA, Inc.	GRN 473	Purified Steviol Glycosides – Reb M (Reb X) Principal Component	General-purpose sweetener, excluding meat, poultry products & infant formulas.
32. GLG Life Tech Corp.	GRN 493	High purity steviol glycosides ≥95%	General-purpose sweetener, excluding meat, poultry products.
33. GLG Life Tech Corp.	GRN 512	High purity Reb M ≥95%	General-purpose sweetener, excluding meat, poultry products & infant formulas.
34. Almendra Limited	GRN 516	Steviol Glycosides with Reb A and Stevioside as the Principal Components	General-purpose sweetener, excluding meat, poultry products & infant formulas.
35. GLG Life Tech Corp.	GRN 536	High purity Reb C and Steviol glycosides with Reb C as the Principal Component	General-purpose sweetener, excluding meat, poultry products & infant formulas.
36. GLG Life Tech Corp.	GRN 548	High purity Reb D	General-purpose sweetener, excluding meat, poultry products & infant formulas.

COMPANY	FDA GRAS IDENTIFIER	MATERIAL IDENTITY	INTENDED FOOD USES
37. Productora Alysa SpA	GRN 555	Steviol Glycosides with Reb A as the Principal Component	General-purpose sweetener, excluding meat, poultry products & infant formulas.
38. PureCircle, Ltd.	GRN 607	Glucosylated steviol glycosides (minimum purity 80%)	Use as a flavoring agent and flavor modifier at levels ranging from 100 to 1,000 ppm
39. PureCircle, Ltd.	GRN 619	Steviol Glycosides ≥95%	General-purpose sweetener, excluding meat, poultry products & infant formulas.
40. Cargill, Inc.	GRN 626	Steviol glycosides (Reb M and Reb D) produced in *Saccharomyces cerevisiae*	General-purpose sweetener
41. DSM Nutritional Products, LLC.	GRN 632	Rebaudioside A from *Yarrowia lipolytica*	General-purpose sweetener, excluding meat, poultry products & infant formulas.
42. Hunan Huacheng Biotech Inc.	GRN 638	High purity steviol glycosides with Reb A as the principal component	General-purpose sweetener, excluding meat, poultry products & infant formulas.
43. GLG Life Tech Corporation	GRN 656	Enzyme-modified steviol glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas.
44. PureCircle USA	GRN 662	Glucosylated steviol glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas.
45. Blue California	GRN 667	Rebaudioside M	General-purpose sweetener, excluding meat, poultry products & infant formulas.
46. Xinghua GL Stevia Co., Ltd	GRN 702	Purified steviol glycosides	General-purpose sweetener
47. Blue California	GRN 715	Rebaudioside D	General-purpose sweetener, excluding meat, poultry products & infant formulas.
48. Shandong Shengiangyuan Biotechnology	GRN 733	Purified steviol glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas.
49. PureCircle Limited	GRN 744	Rebaudioside M	General-purpose sweetener, excluding meat, poultry products & infant formulas.
50. PureCircle Limited	GRN 745	Rebaudioside M	General-purpose sweetener, excluding meat, poultry products & infant formulas.
51. DSM Food Specialties/DSM Nutritional Products North America	GRN 759	Steviol glycosides consisting primarily of Rebaudioside M from *Yarrowia lipolytica*	Flavor and general-purpose sweetener
52. Sichuan Ingia Biosynthetic Co., Ltd.	GRN 764	Rebaudioside D	General-purpose sweetener, excluding meat, poultry products & infant formulas
53. Cargill, Inc.	GRN 768	Stevia leaf extract	General-purpose sweetener, excluding meat, poultry products & infant formulas
54. Tate and Lyle	GRN 780	Rebaudioside M	General-purpose sweetener, excluding meat, poultry products & infant formulas
55. Steviana Bioscience (Suzhou) Inc.	GRN 795	Purified steviol glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas
56. Sichuan Ingia Biosynthetic Co., Ltd.	GRN 799	Rebaudioside M	General-purpose sweetener, excluding meat, poultry products & infant formulas

[a] This table was derived, in part, from McQuate (2011).

[b] The name of this company is now GLG Life Tech Corporation.

[C] GRN 790, submitted by GLG Life Tech Corporation, regarding steviol glycosides (minimum purity 95%), was filed by FDA and is presently under review; GRN 812, Amyris, Inc., regarding Rebaudioside M, was filed by FDA and is presently under review.

In addition, the Flavor and Extract Manufacturers Association (FEMA) has included several steviol glycosides preparations on their GRAS lists as shown in Table 10-2.

Table 10-2. FEMA GRAS Status for Steviol Glycoside Preparations

STEVIOL GLYCOSIDES PREPARATION	FEMA NUMBER	REFERENCE
Rebaudioside A	4601	Smith et al. (2009)
Rebaudioside C; dulcoside B	4720	Leffingwell (2011)
Glucosyl steviol glycosides; enzymatically modified stevia extract	4728	Leffingwell and Leffingwell (2014); Marnett et al. (2013)
Stevioside	4763	Leffingwell and Leffingwell (2014); Marnett et al. (2013)
Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 60%	4771	Marnett et al. (2013)
Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 80%	4772	Marnett et al. (2013)
Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside C 30%	4796	Cohen et al. (2015)
Steviol glycoside extract, *Stevia rebaudiana,* Rebaudioside A 22%	4805	Cohen et al. (2015)
Steviol glycoside extract, *Stevia rebaudiana* Rebaudioside C 22%	4806	Cohen et al. (2015)

b. Canadian Regulatory History

On September 18, 2009, based on a review of the international regulation of *Stevia rebaudiana* and the clinical evidence for safety and efficacy, the Natural Health Products Directorate, Health Canada (2009) adopted the following guidelines for the use of *stevia* and steviol glycosides in Natural Health Products (NHPs) (Health Canada, 2009). The revised recommendation for the maximum limit for steviol glycosides in NHPs is in accordance with the full acceptable daily intake (ADI) of 4 mg steviol per kg bw established by JECFA (WHO, 2008).

On November 30, 2012, Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods (Health Canada, 2012). In March 2014, Health Canada updated the List of Permitted Sweeteners (Lists of Permitted Food Additives) to include steviol glycosides in applications as a table-top sweetener and as an ingredient in a variety of foods, beverages, baked goods, meal replacement bars, condiments, and confectionary and gums (Health Canada, 2014). On January 15, 2016, Health Canada approved the use of Reb M for use as a high-intensity sweetener under the same conditions as the previously approved steviol glycosides (Health Canada, 2016).

Most recently, Health Canada's Food Directorate has updated its List of Permitted Sweeteners to allow for the use of steviol glycosides as a sweetener in 'unstandardized snack bars,' including granola bars, cereal bars, fiber bars, and protein isolate-based bars (Health Canada, 2017b).

Health Canada (2017a) also modified the List of Permitted Sweeteners to include "all the steviol glycosides in the Stevia rebaudiana Bertoni plant (stevia plant)."

c. European Regulatory History

The Joint Expert Committee on Food Additives (JECFA) reviewed steviol glycosides at its 51st, 63rd, 68th and 73rd meetings. In 2000, JECFA published the original review on steviol glycosides (WHO, 2000). JECFA established a temporary ADI of 0-2 mg per kg (on a steviol basis) at its 63rd meeting (WHO, 2006). Additionally, JECFA finalized food grade specifications (FAO, 2007), although they were subsequently updated in 2008 (FAO, 2008) and 2010 (FAO, 2010) (see below). At the 69th meeting, the temporary status of the ADI was removed, and the ADI was raised to 0-4 mg per kg bw per day (on a steviol basis) as a result of the JECFA review of more recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

In early 2009, a number of parties, including the government of Australia and the Calorie Control Council, submitted a request to the Codex Committee on Food Additives in which it was proposed that the JECFA specifications for steviol glycosides should be modified to allow inclusion of rebaudioside D and rebaudioside F as specifically named acceptable glycosides that would be considered as part of the minimum 95% steviol glycosides composition (CCFA, 2009). This proposed modification was endorsed by the Codex Alimentarius Committee in July 2009; it was on the agenda for discussion at the JECFA Meeting in June, 2010 (FAO/WHO, 2009), and JECFA subsequently took final action in approving the modified steviol glycosides specifications to include rebaudioside D and rebaudioside F (FAO, 2010).

In 2008, Switzerland's Federal Office for Public Health approved the use of stevia as a sweetener citing the favorable actions of JECFA (Health, 2008). Subsequently, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009).

In light of JECFA's 2008 findings, and in response to a June 2008 request by the European Commission for European Food Safety Authority (EFSA) to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from three petitioners, EFSA reexamined the safety of steviol glycosides (EFSA, 2010). After considering all the data on stability, degradation products, metabolism and toxicology, the EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg per bw per day, which is similar to JECFA's determination.[12] In addition, on May 25, 2011, EFSA published a determination that the daily dietary intake for use of rebaudioside A as a flavoring substance in a variety of foods would be less than the ADI for steviol glycosides (EFSA, 2011a). In

[12] From a historical perspective, it is noted that the UK's Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food on September 24, 1998 rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made" (MAFF, 1998). In 1999, the Scientific Committee on Food for the European Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves" (EuropeanCommission, 1999a). In another opinion also dated June 17, 1999, the Committee also reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data" (EuropeanCommission, 1999b).

2014, EFSA evaluated extending the use of steviol glycosides as ingredients in food categories to include coffee, tea, and herbal and fruit infusions (assessed at 10 mg per L steviol glycosides). Exposure estimates were lower than those determined by the Panel in 2011 due to available data, and remained below the ADI of 4 mg per kg bw per day, with the exception of toddlers from one country at the 95th percentile exposure level of 4.3 mg per kg bw per day (EFSA, 2014). More recently, exposure estimates, based on maximum permitted levels (MPLs) and proposed use levels increased to 29 mg per L steviol glycosides, were found to have a "negligible" impact on dietary intake for all population groups, with the mean exposure estimate below the ADI of 4 mg per kg bw per day, with the exception of toddlers from one country at the 95th percentile exposure level of 4.3 mg per kg bw per day. The EFSA panel concluded that "dietary exposure to steviol glycosides (E 960) is similar to the exposure estimated in 2014 and therefore does not change the outcome of the safety assessment" (EFSA, 2015).

The appropriate European regulatory bodies, including JECFA and the EFSA, have now agreed that steviol glycosides are safe for all populations to consume and are a suitable sweetening option for diabetics. Effective December 2, 2011, the European Union (EU) approved their use as food additives (EU, 2011). In March 2016, the EU approved the use of steviol glycosides in mustard (Michail, 2016).

Most recently, an amendment to the EU food additives regulation 231/2012, which became active on November 3, 2016, removed the previous requirement for stevia blends to contain at least 75% Reb A or stevioside. In addition, the updated regulation ---(EU) 2016/1814---now permits the following steviol glycosides in stevia blends: stevioside, rebaudiosides A, B, C, D, E, F and M, steviolbioside, rubusoside, and dulcoside (Searby, 2016).

The EFSA Panel of Food Additives and Nutrient Sources reviewed an application for glucosylated steviol glycoside preparations for use as a new food additive. The Panel concluded that the data supplied by the applicant were "insufficient to assess the safety" of the glucosylated steviol glycosides preparation. It should be noted that no safety concerns were raised by the EFSA Panel, and that their decision was based on the "limited" data provided in the dossier submitted by the applicant (EFSA, 2018).

d. Asian Regulatory History

As of May 2010, the government of Hong Kong amended its food regulations to allow the use of steviol glycosides as a permitted sweetener in foods (Safety, 2010). This action followed in the aftermath of the detailed safety evaluation and favorable findings as reported by JECFA.

The international community continued to exhibit much interest in the food uses of steviol glycosides, with additional advances reported in early July 2011. The Codex Alimentarius Commission has adopted proposed maximum use levels for steviol glycosides in all major food and beverage categories, and this action was expected to favorably influence authorizations of stevia uses in India, Indonesia, Thailand, and the Philippines (FoodNavigator, 2011). An article

published online by FoodNavigator (2013) states the following: “with approvals now in Vietnam, the Philippines, Malaysia, Singapore and Thailand, Indonesia is the only [Southeast Asian nation] where stevia hasn’t been given the rubber stamp” (Whitehead, 2013). Furthermore, the International Alliance of Dietary/Food Supplement Associations (IADSA) reported that the Codex Alimentarius Commission agreed to adopt the use of steviol glycosides for addition to chewable food supplements as had been requested by IADSA (NewHope360, 2011).

The Food Safety and Standards Authority of India (FSSAI) convened on September 20, 2012, at which time they approved the use of steviol glycosides as a non-nutritive sweetener in a variety of foods. The FSSAI specified that: the steviol glycosides must meet the specifications and purity as established by JECFA; table top sweetener tablets may contain 7 mg of steviol equivalents per 100 mg carrier/filler, as well as established maximum use levels specific to 11 distinct food categories including dairy, beverage, and chewing gum applications (FSSAI, 2012).

Since December 10, 2012, over thirty registrations have been granted by FDA Philippines to stand-alone steviol glycosides sweeteners or foods containing steviol glycosides as ingredients, including: FR-104390, Steviten Light Brand Steviol Glycosides 95% Sweetener Powder; FR-109427, Del Monte Pineapple Chunks in Extra Light Syrup Reduced Calorie with Steviol Glycosides from Stevia; FR-101120, Diebetamil Zero Calorie Sweetener with Stevia (stick pack); and FR-102127, Sawayaka Stevia Sweetener (1 g sticks) (Philippines, 2014).

Steviol glycosides are also listed under INS number 960 in the Food Additives Permitted Under the Singapore Food Regulations document prepared by the Agri-Food & Veterinary Authority (AVA) of Singapore (AVA, 2014).

e. Other Regulatory History

In 2008, the Food Standards Australia New Zealand (FSANZ) completed its evaluation of an application for use of steviol glycosides in foods. FSANZ recommended that the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) amend the Australia New Zealand Food Standards Code to allow the use of steviol glycosides in food (FSANZ, 2008). In December 2010, FSANZ recommended accepting the increased usage levels as requested since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages, and flavored soy beverages up to 200 mg per kg, and in plain soy beverages up to 100 mg per kg (FSANZ, 2011). In a recent risk assessment, FSANZ concluded that the use of Reb M does not pose any “public health and safety issues” (FSANZ, 2015b). In addition, FSANZ proposed to add Reb M to the list of permitted steviol glycosides (FSANZ, 2015a). On January 14, 2016, Reb M was approved for use “as a food additive in accordance with the current permissions for steviol glycosides” (FSANZ, 2016a).

FSANZ called for submissions on permitting all minor steviol glycosides extracted from stevia leaf to be included in the definition of steviol glycosides in the Food Standards Code, noting that "[no] evidence was found to suggest that the proposed changes pose any public health and safety concerns." The submission period ended on December 19, 2016 (FSANZ, 2016b). Subsequently, on February 8, 2017, FSANZ approved a draft variation of the definition of steviol glycosides to include all steviol glycosides present in the *Stevia rebaudiana* leaf (FSANZ, 2017).

Most recently, FSANZ called for comments on the production of Reb M using enzymes derived from genetically modified yeast (*Pichia pastoris*). The comment period closed on August 31, 2018 (FSANZ, 2018b). Subsequently, on October 31, 2018, FSANZ approved a draft variation to include a reference to the production method (FSANZ, 2018a).

On September 10, 2012, the South African Department of Health issued an amendment to labeling regulations indicating: "in the case of the sweetener steviol glycosides, it shall be described as 'Steviol Glycosides' or 'Steviol Extract.'" On the same date, steviol glycosides were added to the List of Permissible Sweeteners.

Appendix 11 Summary of Published Safety Reviews

1. Summary of JECFA Reviews

At an early review during its 51st meeting, JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

> The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of human metabolism of stevioside and steviol were available. In addition, data on long-term toxicity and carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro.*
>
> In view of the absence of information for the elaboration of specifications for stevioside and since the evaluation of the available toxicological data revealed several limitations, the Committee was unable to relate the results of the toxicological investigations to the commercial product and could not allocate an ADI to stevioside.
>
> Before reviewing stevioside again, the Committee considered that it would be necessary to develop specifications to ensure that the material tested was representative of the commercial product. Further information on the nature of the substance that was tested, data on the metabolism of stevioside in humans and the results of suitable *in vivo* genotoxicity studies with steviol would also be necessary.

Subsequently, additional data were generated on the metabolism of steviol glycosides and submitted to JECFA. This information suggested that the common steviol glycosides are converted to steviol by intestinal bacteria and then rapidly converted to glucuronides that are excreted. The committee now had a molecular basis to become comfortable with new toxicology studies on test materials that consisted of variable composition but were relatively high purity mixtures of the common steviol glycosides. The new information also revealed that in *in vitro* studies, steviol is mutagenic, while in *in vivo* conditions, it is not mutagenic. The committee became convinced that purified steviol glycosides did not impair reproductive performance, as did crude preparations of stevia, and that there were sufficient chronic studies in rats with adequate no observed effect levels (NOEL) that could support a reasonable ADI in the range of doses that would be encountered by the use of steviol glycosides as a sugar substitute. However, JECFA wanted more clinical data to rule out pharmacological effects at the expected doses. The following excerpt was taken from the report of the 63rd meeting (WHO, 2006):

> The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.

> The NOEL for stevioside was 970 mg per kg bw per day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg per kg bw per day (equivalent to 5–10 mg per kg bw per day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes).
>
> The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg per kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg per kg bw per day (or 383 mg per kg bw per day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.
>
> The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

In 2007, at its 68th meeting, JECFA (WHO, 2007) concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008. Subsequently, sufficient data had been received by JECFA to revise and finalize food additive specifications for steviol glycosides. The Chemical and Technical Assessment report, written after the 2007 meeting, explained the Committee's thinking, which resulted in flexibility in the identity specifications (FAO, 2007).

> In response to the call for data on "stevioside" for the 63rd meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63rd JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.
>
> Noting that the additive could be produced with high purity (at least 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68th JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee recognized that the newly revised specifications would cover a range of compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

In 2008, based on additional clinical studies, at its 69th meeting, JECFA finalized the evaluation of steviol glycosides (WHO, 2008), raised the ADI to 0 – 4 mg per kg bw per day, and removed the "temporary" designation. The summary of the Committee's key conclusions in the final toxicology monograph addendum (WHO, 2009) were stated as follows:

> From a long-term study with stevioside, which had already been discussed by the Committee at its fifty-first meeting, a NOEL of 970 mg per kg bw per day was identified. At its sixty-third meeting, the Committee set a temporary ADI of 0–2 mg per kg bw for steviol glycosides, expressed as steviol, on the basis of this NOEL for stevioside of 970 mg per kg bw per day (383 mg per kg bw per day expressed as steviol) and a safety factor of 200, pending further information. The further information was required because the Committee had noted that stevioside had shown some evidence of pharmacological effects in patients with hypertension or with type 2 diabetes at doses corresponding to about 12.5–25.0 mg per kg bw per day (5–10 mg per kg bw per day expressed as steviol).
>
> The results of the new studies presented to the Committee at its present meeting have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg per kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0–4 mg per kg bw expressed as steviol.
>
> The Committee noted that some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

2. Summary of FSANZ Review of Steviol Glycosides

In 2008, FSANZ completed a review of the safety of steviol glycosides for use as a sweetener in foods. FSANZ concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose, or other parameters in normal, hypotensive, or diabetic subjects at doses up to 11 mg per kg bw per day. FSANZ agreed with JECFA in setting an ADI of 4 mg steviol equivalents per kg bw per day, which was derived by applying a 100-fold safety factor to the NOEL of 970 mg per kg bw per day established by a 2-year rat study (Toyoda et al., 1997). The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al. (2008), which was later published in 2008.

In their draft document, FSANZ also indicated that the new data in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, FSANZ established an ADI of 4 mg per kg bw per day for steviol glycosides as steviol equivalents, derived by applying a 100-fold safety factor to the NOEL of 970

mg per kg bw per day (equivalent to 383 mg per kg bw per day steviol) in a 2-year rat study (FSANZ, 2008). In December 2010, FSANZ recommended accepting the increased usage levels since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg per kg and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).

3. Summary of EFSA Review of Steviol Glycosides

On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or rebaudioside A) as a food additive. Earlier---in 1984, 1989 and 1999---the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener. At the time, the SCF concluded that the use of stevioside was “toxicologically not acceptable” due to insufficient available data to assess its safety. However, in light of JECFA’s 2008 findings, and in response to a June 2008 request by the European Commission, EFSA reevaluated the safety of steviol glycosides as a sweetener.

As both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both glycosides, the EFSA Panel agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides. Considering the available safety data (*in vitro* and *in vivo* animal studies and some human tolerance studies), the EFSA Panel concluded that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic, or associated with any reproductive/developmental toxicity. The EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg per kg bw per day based on the application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet. This is equal to 967 mg stevioside per kg bw per day (corresponding to approximately 388 mg steviol equivalents per kg bw per day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that the ADI could possibly be exceeded by European consumers of certain ages and geographies at the maximum proposed use levels.

Recently, EFSA (2011b) revised its exposure assessment of steviol glycosides from its uses as a food additive for children and adults, and published the reduced usage levels in 16 foods by a factor of 1.5 to 3, with no changes for 12 food groups. Additionally, 15 other foods were removed, mainly within the category of desserts and other products, while 3 new food uses were added. The mean estimated exposure to steviol glycosides (equivalents) in European children (aged 1-14 years) ranged from 0.4 to 6.4 mg per kg bw per day and from 1.7 to 16.3 mg per kg bw per day at the 95th percentile. A correction was considered to be necessary for the consumption of non-alcoholic flavored drinks (soft drinks) by children, and the corrected exposure estimate at the 95th percentile for children ranged from 1.0 to 12.7 mg per kg bw per day. For adults, the mean and 97.5th percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg per kg bw per

day, respectively. Non-alcoholic flavored drinks (soft drinks) are the main contributors to the total anticipated exposure to steviol glycosides for both consumer categories. For high consumers, EFSA noted that revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg per kg bw (steviol equivalent).

In addition, EFSA (2011a) recently accepted rebaudioside A as a flavoring agent in a variety of foods. EFSA reviewed the available safety data on rebaudioside A and agreed that the ADI of 4 mg per kg bw per day established for steviol glycosides applied also to rebaudioside A in a purified form. The dietary intake for use as a flavoring agent was calculated by two different methods, and EFSA determined that the worst-case exposure would be 10,888 microgram per person per day, which is equivalent to 181 microgram rebaudioside A per kg bw per day, for a person weighing 60 kg. This corresponds to a daily intake of 60 microgram steviol per kg bw per day, using a conversion factor of 0.33 for converting the amount of rebaudioside A into steviol equivalents.

4. Other Published Reviews

Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008; Geuns et al., 2003a; Huxtable, 2002). Four additional reviews have appeared on the toxicology and biological activity of stevia extracts and steviol glycosides (Yadav and Guleria, 2012; Brown and Rother, 2012; Brahmachari et al., 2011; Chatsudthipong and Muanprasat, 2009). In reviewing these studies, caution is warranted since these reviews do not differentiate well between studies on crude stevia extract and purified steviol glycosides. In addition, many of the reviewed studies on biological activity used routes of administration other than oral, and they may have used doses that are much higher than expected dietary exposures of steviol glycosides as a sweetener. In a letter to the editor of the Journal of Pharmacology and Therapeutics, Roberts and Munro (2009) criticized the Chatsudthipong and Muanprasat (2009) review with some important points that are applicable in general to these four reviews. Important excerpts from this letter are as follows:

> “It is well established that some stevia extracts are crude mixtures that contain multiple components of the stevia leaf, including those components that do not provide a sweet taste. These mixtures also vary considerably in quality, purity, and composition. Therefore, it is not surprising that sometimes these crude and uncharacterized materials may contain substances that possess some degree of pharmacologic activity, but any such effects cannot be attributed specifically to the steviol glycosides. In contrast to studies conducted with less pure steviol glycoside preparations, studies conducted with purified preparations do not indicate any evidence of pharmacological effects.”

> “The authors consistently cite pharmacological, toxicological, and biochemical effects from in vitro studies or from studies in which animals were dosed intravenously (e.g., Melis, 1992 a,b,c). Steviol glycosides are hydrolyzed completely by the gut microflora to steviol prior to absorption, with no systemic absorption of the glycone form following oral exposure. Therefore, the results of in vitro and intravenous, intraperitoneal, or subcutaneous dosing studies of the glycone form are not relevant to the safety of steviol glycosides consumed orally.”

> "Collectively, the report of Chatsudthipong and Muanprasat (2009) is incomplete and lacking discussion of key studies of the safety of stevioside and rebaudioside A. It focuses on alleged effects of stevia and steviol glycosides of low or unknown purity, fails to consider the route of exposure in relation to metabolism and safety assessment and does not include recent opinions expressed by worldwide regulatory authorities affirming the safety of purified forms of stevioside and rebaudioside A as a food ingredient."

Most recently, Urban et al. (2015) reviewed the potential allergenicity of steviol glycosides. The authors noted that: "hypersensitivity reactions to stevia in any form are rare" and concluded that current data do not support claims that steviol glycosides are allergenic. In addition, the authors stated that there is "little substantiated scientific evidence" to warrant consumer warning labels for highly purified stevia extracts (Urban et al., 2015).

Appendix 12 Summary of Studies on Steviol Glycosides Preparations That Are Primarily Rebaudioside A

Safety Data on Rebaudioside A[13]

Since 2008, several well-designed toxicology studies that followed the current regulatory and scientific guidelines for such studies have been reported on purified rebaudioside A, although it is uncertain whether or not these studies were considered by JECFA during its 2008 deliberations. These recent investigations included additional subchronic studies in rats and one in dogs, mutagenicity studies, reproduction and developmental studies in rats, and comparative pharmacokinetic studies with stevioside in rats and humans, as well as additional clinical studies. These studies confirm that rebaudioside A is metabolized similarly to other steviol glycosides, and they exhibited an absence of toxicological effects in the key studies reviewed by JECFA. It should be noted that rebaudioside A, as the steviol glycoside with high sweetness intensity and relatively high prevalence in the stevia leaves, remains an active topic of scientific research. For example, a study found in a recent literature search examined the anti-hyperglycemic activity of rebaudioside A in diabetic rats (Saravanan and Ramachandran, 2012). These investigators found that the effects of streptozotocin-induced diabetes on glucose and insulin levels were at least partially reversed in a dose-dependent manner with oral administration of rebaudioside A at doses in the range of 50-200 mg per kg bw. The doses used are 10-40 times higher than expected from the use of rebaudioside A as a sweetener. The known anti-hyperglycemic activity of steviol glycosides led JECFA to require clinical studies at reasonably high doses to show that—at levels used in food—there would be no effect on glucose homeostasis or blood pressure in human consumers. The clinical studies described below on rebaudioside A (Maki et al., 2008a; Maki et al., 2008b) the lack of these pharmacological effects of rebaudioside A at expected levels of consumption.

1. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

Studies investigating the ADME of extracts from stevia are available on stevioside, Reb A, and other steviol glycosides. Data evaluating the absorption and fate of these extracts from various animal species and humans indicate that one can extrapolate these results from rats to humans. Stevioside is metabolized to steviol *via* intestinal microflora, and the absorption of stevioside after oral administration has been shown to be very low (Koyama et al., 2003b; Geuns et al., 2003b; Geuns et al., 2003a).

Studies investigating the hydrolysis of steviol glycosides by intestinal microflora have demonstrated that both stevioside and Reb A are hydrolyzed to steviol following *in vitro* incubation with various cecal microflora (Wingard Jr et al., 1980; Hutapea et al., 1997; Gardana et al., 2003; Geuns et al.,

[13] Questions about the safety of rebaudioside A were previously raised by Huxtable (2002), and Kobylewski and Eckhert (2008). Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels, have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS notifications 278, 287, 303, and 304.

2003a). In addition, the *in vitro* hydrolysis of Reb A to steviol was found to be slower than that of stevioside (Koyama et al., 2003b), which is thought to be partly due to the presence of one additional glucose moiety and to differences in structural complexities. Koyama et al. (2003b) suggest that the major pathway for Reb A is conversion to stevioside with a minor pathway of conversion to Reb B prior to being ultimately converted to steviol. Stevioside is further converted to steviolbioside, steviolmonosides, and finally steviol, with glucose being released with each subsequent hydrolysis.

In three recently completed studies, absorption and fate of rebaudioside A were systematically investigated in rats and humans.

For comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A, toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats (Roberts and Renwick, 2008). Orally administered single doses of the radiolabeled compounds were extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A.

Roberts and Renwick (2008) identified free steviol (82 to 86%), steviol, glucuronide (10 to 12%), and two unidentified metabolites (5-6%) in rat plasma following treatment with either stevioside or Reb A eight hours post-oral administration. A comparable pharmacokinetic profile was noted following oral treatment of rats with radiolabeled Reb A or stevioside, with the time of maximum plasma concentration (T_{max}) for radioactivity ranging between 2 and 8 hours. In comparison, steviol T_{max} for plasma was noted within 30 minutes of oral administration. All plasma samples had similar metabolite profiles; the predominant radioactive component in all samples was steviol, with lower amounts of steviol glucuronide(s) and low levels of one or two unidentified metabolites. It is believed that this delay between the occurrence of radioactivity in the plasma and time of administration of steviol glycosides is due to the fact that the Reb A and stevioside are first cleaved to steviol before absorption.

Within 72 hours of administration, elimination of radioactivity from plasma was essentially complete. Following elimination in the bile, steviol is available to be released again from its conjugated form by microflora activity and may enter enterohepatic circulation. Consequently, free and conjugated steviol are secreted in the feces along with any unhydrolyzed fraction of the administered glycosides. Following Reb A treatment, significant amounts of unchanged rebaudioside A (29% in males and 19% in females) and stevioside (3% in males and 4% in females) were excreted in the feces. Following oral stevioside administration, unchanged stevioside was excreted in rat feces. Other unidentified metabolites are also present in fecal samples of rats treated with either glycoside. Rebaudioside A, stevioside, and steviol were metabolized and excreted rapidly, with ~60% of the radioactivity eliminated in the feces within 48 hours. Urinary excretion accounted for less than 2% of the administered dose for all compounds in both intact and bile duct-cannulated rats, and the majority of the absorbed dose was excreted *via* the bile. After administration of the compounds to intact and bile duct-cannulated rats, radioactivity

in the feces was present primarily as steviol. The predominant radioactive compound detected in the bile of all cannulated rats was steviol glucuronide (Roberts and Renwick, 2008).

In summary, Roberts and Renwick (2008) found that steviol was the predominant component found in plasma samples after oral administration of Reb A, stevioside, and steviol in rats. Lower amounts of steviol glucuronide(s) and one or two unidentified metabolites were also found. The majority of all samples were found to be excreted rapidly---primarily in the feces---within 48 hours. This is in agreement with the previous *in vitro* hydrolysis data that indicated that both Reb A and stevioside are metabolized to steviol by intestinal microflora. The predominant compound detected in the bile was steviol glucuronide, while the prominent material in the intestine was steviol, which the authors suggest indicates that deconjugation occurs in the lower intestine. The authors concluded that the overall data on toxicokinetics and metabolism indicate that rebaudioside A and stevioside are handled in an almost identical manner in the rat after oral dosing.

In a randomized, double blind, cross-over study in healthy male subjects, Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside. Following administration of rebaudioside A or stevioside, steviol glucuronide appeared in the plasma of all subjects, with median T_{max} values of 12.0- and 8.00-hours post-dose, respectively. Steviol glucuronide was eliminated from the plasma, with similar $T_{1/2}$ values of approximately 14 hours for each compound. Administration of rebaudioside A resulted in a significantly (~22%) lower steviol glucuronide geometric mean C_{max} value (1,472 ng per mL) than administration of stevioside (1,886 ng per mL). The geometric mean AUC_{0-t} value for steviol glucuronide after administration of rebaudioside A (30,788 ng*hr per mL) was approximately 10% lower than after administration of stevioside (34,090 ng*hr per mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72-hour collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both rebaudioside A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide, indicating rapid first-pass conjugation prior to urinary excretion. Only a small amount of steviol was detected in urine (rebaudioside A: 0.04%; stevioside: 0.02%). The investigators concluded that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in humans, with steviol glucuronide excreted primarily in the urine and steviol in the feces. No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety, or vital signs (Wheeler et al., 2008).

Another pharmacokinetic investigation was done as a toxicokinetic (TK) phase of a dietary study to determine the potential of rebaudioside A toxicity in rats at levels up to 2,000 mg per kg bw per day (Sloter, 2008a). Extremely low levels of rebaudioside A and total steviol were detected in peripheral blood of rats during daily administration of 2,000 mg per kg bw per day of rebaudioside A, with mean plasma concentrations of approximately 0.6 and 12 μg per mL, respectively. Estimates of absorbed dose for rebaudioside A and total steviol were approximately 0.02% and 0.06%, respectively, based on the amounts measured in urine collected over 24 hours in

comparison to daily administered dietary dose to rats. Mean fecal rebaudioside A and measured hydrolysis products, expressed as Total Rebaudioside A Equivalents, compared to daily administered dose results in an estimated dose recovery of approximately 84%.

2. Subchronic Toxicity Studies

Curry and Roberts (2008) reported the results of two repeat dose studies of rebaudioside A in Wistar rats. The results of these investigations suggest that administration of rebaudioside A to Han Wistar rats at dietary concentrations of up to 100,000 ppm (9,938 and 11,728 mg per kg bw per day for males and females, respectively) for 4 weeks, or 50,000 ppm (4,161 and 4,645 mg per kg bw per day for males and females, respectively) for 13 weeks, did not present any evidence of systemic toxicity. In the 4-week study, rebaudioside A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000, and 100,000 ppm to male and female rats. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were fed diets containing rebaudioside A at dietary concentrations of 0, 12,500, 25,000, and 50,000 ppm. In high-dose male and female groups, reductions in body weight gain attributable to initial taste aversion and lower caloric density of the feed were observed. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of rebaudioside A *via* the liver. All other hepatic function test results and liver histopathology were within normal limits. No significant changes in other clinical pathology results, organ weights, and functional observational battery test results were noted. Macroscopic and microscopic examinations of all organs were unremarkable with respect to treatment-related findings. The NOAEL in the 13-week toxicity study was considered to be 50,000 ppm, or approximately 4,161 and 4,645 mg per kg bw per day in male and female rats, respectively (Curry and Roberts, 2008).

In another 90-day dietary admix toxicity study, effects of rebaudioside A (99.5% purity) at target exposure levels of 500, 1,000, and 2,000 mg per kg bw per day were tested in Crl:CD(SD) rats (Nikiforov and Eapen, 2008; Eapen, 2007). Each group consisted of 20 animals per sex. No treatment related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters were noted. There were no treatment-related macroscopic, organ weight or microscopic findings. Significantly lower body weight gains were noted in the 2,000 mg per kg bw per day group in males but not females. At the end of the dosing period, the body weight in males was 9.1% lower than the control group. Due to the small magnitude of difference from the control group value, the investigators did not consider this result to be adverse. The decrease was most likely due to the large proportion of the diet represented by the test material. The NOAEL was determined as ≥ 2,000 mg per kg bw per day.

A 6-month dietary toxicity study in Beagle dogs (4 per sex per group) was conducted to investigate the potential adverse effects of rebaudioside A (97.5% purity) at dosage levels of 0, 500, 1,000, or 2,000 mg per kg bw per day (Eapen, 2008). There were no unscheduled deaths during the course of the study. No treatment-related clinical observations were noted. Administration of rebaudioside A did not affect home cage, open field observations and functional observations and

measurements. No differences in hematology findings, serum chemistry findings, or urinalysis findings between the groups were noted. Additionally, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. The investigators concluded that no systemic toxicity of rebaudioside A was observed at dosage levels up to 2,000 mg per kg bw per day and the assigned NOAEL was ≥ 2,000 mg per kg bw per day.

In addition, a 90-day subchronic toxicity study was conducted in Sprague-Dawley rats using fermentation-derived Rebaudioside A, where no systemic or local toxicity was observed in rats dosed at 500 to 2,000 mg per kg bw per day. All test animals survived to scheduled necropsy (Rumelhard et al., 2016).

3. Mutagenicity Studies

In a set of *in vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage, and deoxyribonucleic acid (DNA) strand breakage, rebaudioside A consistently and uniformly revealed negative results (Pezzuto et al., 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002). These studies were critically reviewed by Brusick (2008). JECFA also reviewed an unpublished chromosome aberration assay of rebaudioside A in cultured mammalian cells (Nakajima, 2000a) and did not find increases in chromosome aberrations.

Additionally, FDA also reviewed three unpublished studies on rebaudioside A, including a bacterial mutagenicity study (Wagner and Van Dyke, 2006), a mouse lymphoma study (Clarke, 2006), and a mouse micronucleus study (Krsmanovic and Huston, 2006), submitted by Merisant as part of the GRAS Notification. All three studies demonstrated lack of mutagenic or genotoxic activity. Furthermore, Williams and Burdock (2009) also reported lack of genotoxicity in another set of published studies that included *in vitro* mutagenicity assays with *Salmonella, E. coli,* and mouse lymphoma cells. These investigators also reported lack of *in vitro* clastogenic effects in Chinese hamster V79 cells, and the absence of *in vivo* effects in a mouse micronucleus assay and a rat study for unscheduled DNA synthesis.

The recent evaluation of fermentation-derived rebaudioside A demonstrated a similar safety profile to plant-derived rebaudioside A. Rumelhard et al. (2016) reported that fermentation-derived rebaudioside A was not mutagenic in the bacterial reverse mutation assay, nor was it found to be clastogenic or aneugenic in the *in vitro* micronucleus assay. The similarity of the safety profile observed between plant-derived and fermentation-derived rebaudioside A further supports the applicability of the safety assessments to other steviol glycoside preparations.

The key mutagenicity testing results for rebaudioside A are summarized in Table 12-1.

Table 12-1. Mutagenicity & Genotoxicity Studies on Rebaudioside A

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
Bacterial Mutagenicity	5 *Salmonella* strains with & without exogenous metabolic activation system	Reb A	99.5	1.5, 5.0, 15, 50, 150, 500, 1,500 & 5,000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Bacterial Mutagenicity	4 *Salmonella* strains & 1 *E. coli* strain with & without exogenous metabolic activation system	Reb A	95.6	Up to 5,000 µg per plate	No mutagenic response	Williams and Burdock (2009)
Bacterial Mutagenicity	4 *Salmonella* strains & 1 *E. coli* strain with and without exogenous metabolic activation system	Fermentation-derived Reb A	≥ 95%	Up to 5,000 µg per plate	No mutagenic response	Rumelhard et al. (2016)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence & presence of exogenous metabolic activation system	Reb A	99.5	Cloning conc. of 500, 1,000, 2,000, 3,000, 4,000 & 5,000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence & presence of exogenous metabolic activation system	Reb A	95.6	Up to 5,000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Human Lymphocytes	Human lymphocytes in absence & presence of exogenous activation system	Fermentation-derived Reb A	≥ 95%	Up to 5,000 µg/mL	Not clastogenic or aneugenic	Rumelhard et al. (2016)
Chromosome Aberration	Human lymphocytes in absence & presence of exogenous metabolic activation system	Reb A	95.6	Up to 5,000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study in groups of 5 male & 5 female ICR mice	Reb A	99.5	500, 1,000 & 2,000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
Mouse Micronucleus	Micronucleus study in groups of 5 male & 5 female NMRI mice	Reb A	95.6	Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	Unscheduled DNA synthesis in one group of 4 Wistar rats	Reb A	95.6	Up to 2,000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52%; Reb A, 22%	250 – 2,000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2,000 mg/kg bw/ day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

NS = Not specified

[a] Sacrificed at 3 hours and 24 hours

[b] With or without metabolic activation (source not specified in original monograph)

[c] Sacrificed at 30 hours after 2nd administration

4. Reproductive & Developmental Toxicity Studies

In a two-generation reproductive toxicity study, rebaudioside A (97% purity) at 0, 7,500, 12,500, and 25,000 ppm was administered in diet to male and female Han Wistar rats (Curry and Roberts, 2008). Administration of rebaudioside A was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. Similarly, administration of rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the F_0 or F_1 generations. The survival and general condition of the F_1 and F_2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by rebaudioside A treatment. The NOAEL for reproductive effects was 25,000 ppm, and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm, or 2,048 to 2273 mg per kg bw per day (the highest dose tested).

The results from two unpublished studies with rebaudioside A (Sloter, 2008a; Sloter, 2008b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30 per sex per group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter, 2008a). For the F_0 and F_1 generations, rebaudioside A doses were 0, 500, 1,000, and 2,000 mg per kg per day. At initiation of study, F_0 animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F_1 generation following weaning [beginning on postnatal day (PND) 21]. The F_0 and F_1 males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F_0 and F_1 females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females as evaluated by estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints. Both for parental systemic and reproductive toxicity, a dose level ≥ 2,000 mg per kg bw per day (highest dose administered) was assigned to be the NOAEL.

In an embryo/fetal developmental toxicity study in rats (Sloter, 2008b), effects of rebaudioside A administered *via* gavage were investigated. Rebaudioside A administration did not affect intrauterine growth and survival, and there were no test article-related fetal malformations or developmental variations at any dosage level. In the absence of maternal or developmental toxicity, a dose level ≥ 2,000 mg per kg bw per day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

5. Clinical Studies on Rebaudioside A

In a four week randomized, double-blind, placebo controlled trial, hemodynamic effects of rebaudioside A, at a dose of 1,000 mg per day rebaudioside A (97% purity) or placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP), were investigated (Maki et al., 2008a). Subjects were predominantly female (76% rebaudioside A and 82% placebo) with a mean age of ~41 (range 18 to 73) years. At baseline, mean resting, seated SBP/DBP was 110.0/70.3 mm Hg and 110.7/71.2 mm Hg for the rebaudioside A and placebo groups, respectively. Compared with placebo, administration of rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The investigators concluded that consumption of 1,000 mg per day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

In another trial, effects of 16 weeks of consumption of 1,000 mg per person per day rebaudioside A (97% purity, n = 60) were compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki et al., 2008b). Changes in glycosylated hemoglobin levels did not differ significantly between the rebaudioside A (0.11 ± 0.06%, mean ± standard error) and placebo (0.09 ± 0.05%; $p = 0.355$) groups. Similarly, no significant ($p > 0.05$ for all) changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose (7.5 ± 3.7 mg per dL and 11.2 ± 4.5 mg per dL), insulin (1.0 ± 0.64 µU per mL and 3.3 ± 1.5 µU per mL), and C-peptide (0.13 ± 0.09 ng per mL and 0.42 ± 0.14 ng per mL) were noted. No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess versus placebo. Based on these results, the investigators suggested that chronic use of 1,000 mg per person per day rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

6. Safety of Rebaudioside A

There have been a significant number of studies regarding the safety and toxicity of rebaudioside A, including many that have been published since the two initial GRAS notifications were submitted to FDA by Cargill (GRN 253) and Merisant (GRN 252). These, and some other unpublished studies, formed the basis of the two initial GRAS notifications to FDA by Cargill (GRN 253) and Merisant (GRN 252). Prior to this, a limited number of toxicology studies specifically on rebaudioside A were conducted. Even before these new studies were completed, and as noted in the previous section, JECFA concluded that 7 (which was later expanded to 9) common steviol

glycosides are deemed to be safe for use as sweetener preparations when present in any combination, as long as a combined purity of 95% or more was established.

Since a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on rebaudioside A was to conduct a limited number of well-designed and executed toxicology studies on rebaudioside A itself, and to demonstrate that rebaudioside A is handled pharmacokinetically similarly to stevioside in rats and humans. This approach appears to have been undertaken to justify the JECFA-generated ADI without having to conduct a chronic study in rats with rebaudioside A. Additionally, the Merisant group conducted three mutagenicity assays on rebaudioside A that FDA generally considers to be most predictive for carcinogenicity potential. The Cargill group conducted two clinical studies to assure that rebaudioside A does not have potentially problematic pharmacological effects on blood glucose and blood pressure.

In a review article, Carakostas et al. (2008) summarized the most recent Cargill research program findings on rebaudioside A, as follows:

- Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro*.
- In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes et al., 2007) and was improperly interpreted as a positive response.
- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- While studies with Reb A indicated slight gastrointestinal (GI) absorption of the glycoside *per se*, the predominant metabolic pathway is comparable to that of stevioside and the use of the ADI established by JECFA, which was determined on studies employing stevioside as the main component, can be used as the ADI for rebaudioside A.
- The dietary levels expected from consumption of rebaudioside A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

The consumption estimates described by JECFA, Renwick (2008), and the GRN 252 and GRN 253 Expert Panels very conservatively represent a potential high user of Rebaudioside A if this non-nutritive sweetener becomes widely available in food.

Regarding the available aggregate safety information, multiple qualified entities have concluded that JECFA has critically and extensively evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity, as defined by JECFA specifications, has been properly determined to be 4 mg per kg bw per person as steviol

equivalents, which corresponds to 12 mg per kg bw per day for rebaudioside A, on a dry weight basis. Unwanted pharmacological effects are not likely to occur at this level and, moreover, high consumers of rebaudioside A are not likely to exceed this level. Therefore, the JECFA-derived ADI was adopted as a safe exposure for rebaudioside A and the corresponding food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be generally recognized as safe (GRAS).

JECFA---which is composed of dozens of scientists that are internationally known experts on food ingredient safety---has established ADIs for food ingredients over the last 40 years. Both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of rebaudioside A. The studies were equally well conducted. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of rebaudioside A safety.

The studies conducted by Cargill provided significant insight into the pharmacokinetics of rebaudioside A, while demonstrating clinical safety of rebaudioside A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food. The Merisant notification augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems. Additional mutagenicity and genotoxicity studies have been published on rebaudioside A (Williams and Burdock, 2009). Merisant added a subchronic study in dogs and a teratology study in rats. Both Cargill and Merisant relied on the JECFA ADI for steviol glycosides as determined largely by published chronic studies in rat. Both groups justified the use of the ADI on pharmacokinetic arguments showing the similarity of stevioside and rebaudioside A metabolism and excretion.

Appendix 13 Studies on Principal Metabolite: Steviol

Studies on Principal Metabolite: Steviol

In a number of studies, steviol, the principal mammalian metabolite of stevioside, has been investigated for its safety. The results of these studies are summarized below.

1. Acute Toxicity Studies

The oral LD_{50} of steviol (purity, 90%) in male and female mice and rats was reported to be > 15 grams per kg bw. In this study, only one of 15 animals died within 14 days of administration. The LD_{50} values in hamsters given steviol orally were 5.2 grams per kg bw in males and 6.1 grams per kg bw in females. Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure (Toskulkac et al., 1997).

2. Developmental Toxicity Studies

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1,000 mg per kg bw per day (only 12 animals at the highest dose) by gavage in corn oil on days 6 - 10 of gestation. A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). However, no dose-dependent teratogenic effects were seen. The NOEL was 250 mg per kg bw per day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

3. Mutagenicity & Genotoxicity Studies

In a number of studies mutagenicity and genotoxicity of steviol has been investigated. These studies reviewed by JECFA are summarized in Table 13-1.

Table 13-1. Mutagenicity & Genotoxicity Studies on Steviol

	In Vivo/In Vitro	System	Test Sample Purity	Author Conclusion	Results and Remarks
Sekihashi et al. (2002)[a]	*In Vivo/In Vitro*	Comet Assay	Not reported	Negative	In *in vitro* study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In *in vivo* study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1,000 or 2,000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged. An identical *in vivo* experiment with stevia extract performed, which also gave negative results.
Oh et al. (1999)[b]	*In Vivo?*	Cell Mutation and DNA damage	Not reported	Negative	Steviol gave negative results for cell mutation and DNA damage in cultured cells.
Matsui et al. (1996)[c]	*In Vivo?*	Mutagenicity and Chromosome aberration (Chinese hamster lung fibroblasts)	Not reported	Positive	Gene mutation and chromosomal aberration found in Chinese hamster lung fibroblasts after metabolic activation of steviol. In hamsters, several metabolites of stevioside found that have not been found in rats or humans. Therefore, experimental relevance should be questioned when hamsters are used.
Terai et al. (2002)[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Steviol found to be mutagenic in Aroclor-induced rat liver S9 fraction. 15-oxo-steviol found to be mutagenic at 10% level of steviol. Specific mutagenicity of lactone derivative in presence of S9 mixture 10x lower than that of derivative without S9 mixture.
Temcharoen et al. (1998)[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Mutagenic effects of steviol and/or metabolites found in *S. typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported.
Klongpanichpak et al. (1997)[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Steviol and stevioside inactive in TA strains of *S. typhimurium, E. coli WP2, uvrA/PKM101* and rec assay using *B. subtilis* even when microsomal activated fraction present. Magnitude of increase of these mutations over the control not reported.
Matsui et al. (1996)[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Testing of Southern Blot technique with probe for gpt gene DNA of *E. coli*. The chromosomal DNA of TM677 and steviol-induced TM677 mutants digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA.
Matsui et al. (1996)[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Both	Steviol weakly positive in umu test, either with or without metabolic activation. Steviol negative in reverse mutation and other bacterial assays even in presence of S9 activation.

	In Vivo/In Vitro	System	Test Sample Purity	Author Conclusion	Results and Remarks
Procinska et al. (1991)[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	The direct mutagenic activity of 15-oxo-steviol was refuted.
Compadre et al. (1988)[a]	*In Vitro*	Bacterial Mutagenicity, Mass Spec	Not Reported	Positive	Mass spectral analysis of steviol and analogues under conditions known to produce a mutagenic response. 15-oxo-steviol, a product of the metabolite, 15-alpha-hydroxysteviol was found to be direct-acting mutagen. Magnitude of increase over control in assay not discussed.
Pezzuto et al. (1985)[d]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Using *S. typhimurium* TM677 strain, steviol found to be highly mutagenic in presence of 9000 x g supernatant from livers of Aroclor 1254-pretreated rats. This mutagenicity dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of other metabolites tested was mutagenic. Authors concluded that structural features of requisite importance for the expression of mutagenic activity may include a hydroxy group at position 13 and an unsaturated bond joining the carbon atoms at positions 16 and 17.
Temcharoen et al. (2000)[c]	*In Vivo*	Micronucleus (rat)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Temcharoen et al. (2000)[c]	*In Vivo*	Micronucleus (mouse)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Matsui et al. (1996)[a]	*In Vivo*	Micronucleus (mouse)	Not Reported	Negative	Steviol did not increase number of micronuclei observed in this study.
Temcharoen et al. (2000)[c]	*In Vivo*	Micronucleus (hamster)	90%	Negative	Very high doses (4 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.

[a] Abstract only
[b] As reported in WHO (2006)
[c] As reviewed by Geuns et al. (2003a)
[d] Full article

4. Endocrine Disruption Studies

Shannon et al. (2016) investigated the endocrine disrupting potential of stevioside, rebaudioside A, and steviol in a series of *in vitro* bioassays. Steviol was reported to 1) antagonize progesterone nuclear receptor transcriptional activity; 2) increase progesterone production; and 3) induce an agonistic response on the progesterone receptor of sperm cells (Catsper). While the authors concluded that *Stevia* might not qualify as a safer alternative to sugar or synthetic sweeteners, it is important to note that it is difficult to translate *in vitro* concentrations to local concentrations *in vivo* at the receptor level. Furthermore, no adverse effects were observed in the reproductive studies.

Appendix 14 GRAS Associates Expert Panel Report

The Generally Recognized as Safe (GRAS) Status of the Proposed Uses of Rebaudioside M

January 29, 2019

Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC on behalf of their client, GLG Life Tech Corporation ("GLG"), to evaluate the safety and Generally Recognized as Safe (GRAS) status for GLG's proposed uses of Festeviol™ RM 95 in conventional foods. The members of this Expert Panel† are qualified to serve in this capacity by qualification of scientific training and experience in the safety of food and food ingredients.

Discussion

A significant amount of safety information related to the consumption of steviol glycosides is generally available, and has been discussed in Part 6, as well as Appendices 8-13, of GLG's dossier. First, there is a history of safe consumption of steviol glycosides when used as an ingredient in food products in the U.S., Canada, South America, Europe, Asia, and Australia and New Zealand. Second, a number of experimental studies have investigated the safety of steviol glycosides. The composite evidence from historical safe consumption and experimental studies together demonstrate the safety of Festeviol™ RM 95 for human food consumption.

The majority of the studies reviewed on steviol glycosides and steviol have been discussed in detail in previous GRAS notifications (GRNs), including GRN 536, GRN 548, and GRN 656, which were submitted by GLG.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the gastrointestinal (GI) tract, *per se*; they are converted to steviol by bacteria normally present in the large intestine, and the steviol is absorbed but is rapidly glucosylated and excreted in the urine and feces. It has been well-established experimentally from various published studies that the steviol glycoside molecules are not absorbed from the GI tract (Gardana et al., 2003; Koyama et al., 2003b). The action of bacteria in the large intestine is directly

† Dr. Emmel, Chair of the Expert Panel, is a chemist with substantial food safety experience in addressing steviol glycosides and other food ingredients. Dr. Archer is a microbiologist with extensive experience regarding GRAS issues, including regulatory policies, microbiology, food processing, and toxicology. Prior to his professorship at the University of Florida, Dr. Archer served as Deputy Director, CFSAN, FDA. Dr. Lewis is a biologist with more than 10 years of experience preparing GRAS dossiers. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety and in participating in deliberations of GRAS Expert Panels.

supported by the published study that steviol glycosides can be converted to steviol in the large intestine by normal anaerobic GI flora as demonstrated by an *in vitro* study in fecal homogenates (Koyama et al., 2003a; Renwick and Tarka, 2008). Geuns et al. (2006) measured blood, urine, and fecal metabolites in human subjects who received purified stevioside over 3 days and found steviol glucuronide in blood, urine, and feces samples. The authors concluded that there was complete conversion of stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide.

In vitro metabolism studies have reported that rebaudioside M is hydrolyzed to steviol within 24 hours, much like other steviol glycosides, including Reb A. Metabolism was found to be concentration-dependent and the majority of metabolism occurred within the first 8 hours (PureCircle, 2013b; Purkayastha et al., 2014). These observations support the presumption of safety of rebaudioside M, given the similarities in metabolism with Reb A.

The acceptable daily intake (ADI) for steviol glycosides has been set largely based on a published chronic study in rats (Toyoda et al., 1997) and several published clinical studies show that there are no pharmacological effects in humans at doses several fold higher than the ADI (Barriocanal et al., 2006; Barriocanal et al., 2008; Wheeler et al., 2008). Recently, Roberts et al. (2016) noted in a persuasive argument using a chemical-specific adjustment factor (CSAF) that the ADI could be higher. The toxicity of the metabolite, steviol, has been well-reviewed in the published literature (Geuns et al., 2003a; WHO, 2006; Urban et al., 2013). In addition, FDA has issued “no questions” letters to 56 GRN submissions for steviol glycosides preparations as of December 11, 2018.

The Expert Panel notes that GLG’s manufacturing process for Festeviol™ RM 95 is similar to the bioconversion processes described for GRAS rebaudioside M preparations prepared with genetically-modified *E. coli*, as described in in GRN 745 (PureCircle, 2018b) and GRN 780 (Tate and Lyle, 2018), and for high purity steviol glycosides prepared with genetically-modified *Bacilli*, as described in GRN 375 (Toyo Sugar Refining Co., 2011); GRN 448 (Daepyung, 2012); GRN 607 (PureCircle, 2015); and GRN 662 (PureCircle, 2016).

The updated scientific literature review of steviol glycosides covering the time frame since GRN 780 was submitted through the present revealed no findings raising new safety concerns that would alter the previous GRAS determinations for similar rebaudioside M preparations.

The GRAS Associates Expert Panel convened on behalf of GLG has reviewed the proposed uses for Festeviol™ RM 95. The highest 90th percentile consumption by any population subgroup of Festeviol™ RM 95 was calculated to be approximately 4.95 mg per kg bw per day, which is equivalent to 1.22 mg per kg bw per day steviol equivalents (calculated by a weighted sum estimate) for any population group, on a worst-case scenario basis. This estimated intake value is well below the JECFA ADI of 4 mg per kg bw per day expressed as steviol equivalents. Therefore, Festeviol™ RM 95 is expected to be safe within established allowable limits.

A compelling case can be made that scientific consensus exists regarding the safety of steviol glycosides when of sufficiently high purity. The central role of conversion to steviol and subsequent elimination with these naturally occurring steviol glycosides extends to the manner in which the various steviol glycosides molecules are metabolized and eliminated from the body. While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide consensus does exist in the scientific community to support a GRAS conclusion as evidenced by several publications (Carakostas, 2012; Geuns, 2007; Urban et al., 2013; Waddell, 2011; Williams, 2007; Brusick, 2008) that effectively refute safety concerns expressed by a minority of scientists. In addition, Roberts et al. (2016) suggest that the ADI for steviol glycosides could be as high as 6-16 mg per kg bw per day, which is higher than has been previously accepted by the scientific community, providing the potential for an even more robust safety profile.

In summary, sufficient qualitative and quantitative scientific evidence in the composite is available to support the safety-in-use of GLG's Festeviol™ RM 95 preparation given the following conditions:

- GLG's Festeviol™ RM 95 preparation continues to meet the designated specifications;
- the minimum sweetness intensity GLG's Festeviol™ RM 95 remains unchanged; and
- GLG's Festeviol™ RM 95 is produced in accordance with Current Good Manufacturing Practices (CGMPs).

Conclusion

The Expert Panel critically reviewed the data provided by GLG for their Festeviol™ RM 95 preparation, as well as publicly available published information obtained from peer reviewed journals and other safety assessments prepared by other Expert Panels and well-respected authoritative international regulatory bodies.

The ingestion of GLG's Festeviol™ RM 95 from the intended uses results in intakes that are safe within the limits of established historical use and published safety studies and the widely accepted ADI of 4 mg per kg per day steviol equivalents.

The Expert Panel unanimously concluded that the proposed uses of GLG's Festeviol™ RM 95 preparation, manufactured as described in Part 2.B. of their dossier, and declared within the subject notification meet the FDA definition of safety in that there is "reasonable certainty of no harm under the intended conditions of use" as described herein, and GLG's Festeviol™ RM 95 preparation is GRAS.

Doug Archer, Ph.D.

Kara Lewis, Ph.D.

Katrina Emmel, Ph.D.
Panel Chair

END

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
846	2/27/2019
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A

☐
☐

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person Simon Springett	Position or Title Vice President of Operations		
	Organization *(if applicable)* GLG Life Tech Corporation			
	Mailing Address *(number and street)* 10271 Shellbridge Way, Suite 100			
City Richmond	State or Province British Colombia (BC)	Zip Code/Postal Code V6X 2W8	Country Canada	
Telephone Number 604-285-2602	Fax Number 604-661-8858	E-Mail Address simon.springett@glglifetech.com		
1b. Agent or Attorney *(if applicable)*	Name of Contact Person William J. Rowe	Position or Title President		
	Organization *(if applicable)* GRAS Associates			
	Mailing Address *(number and street)* 27499 Riverview Center Blvd., Suite 212			
City Bonita Springs	State or Province Florida	Zip Code/Postal Code 34134	Country United States of America	
Telephone Number 239-444-1724	Fax Number 239-444-1723	E-Mail Address wrowe@nutrasource.ca		

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Rebaudioside M (>95%)

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than infant formulas and meat and poultry products, at per serving levels reflecting good manufacturing practices and principles, in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that GLG Life Tech Corporation
(name of notifier)

has concluded that the intended use*(s)* of Rebaudioside M (>95%)
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. GLG Life Tech Corporation agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

10271 Shellbridge Way, Suite 100, Richmond, BC V6X 2W8 Canada
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official Agent or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
[redacted]	Katrina Emmel on behalf of William J. Rowe, President	02/19/2019

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Multiple Appendices ---Appendices 1 through 14	